JOURNAL OF AGRICULTURAL RESEARCH

VOL. 33 WASHINGTON, D. C., DECEMBER 15, 1926 NO. 12

SENSES OF THE COTTON BOLL WEEVIL—AN ATTEMPT TO EXPLAIN HOW PLANTS ATTRACT INSECTS BY SMELL[1]

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INTRODUCTION

A thorough and comprehensive investigation of the senses and sense organs of the cotton boll weevil (*Anthonomus grandis* Boh.) will undoubtedly throw light on the means by which the cotton plant attracts this insect. Entomologists have considerable faith in the theory that there is some definite or indefinite relation, or possibly attraction, between insects and their host plants. In regard to some of the insect-pollinated plants it has been proved that the relation is a definite and mutual one, the insects and plants both being benefited; the insects receiving nectar or pollen in exchange for the unintentional labor of cross-pollination. In this case the insects are attracted either by sight or smell, but more probably by both. In other cases, however, the attraction is certainly not mutual, because only the insects profit by the relation. In such cases, how are the insects attracted—by sight or smell, or by some unknown sense? For short distances we believe that either sight or smell would suffice, but boll weevils are apparently able to locate cotton plants from long distances and do not seem to make mistakes in doing so. If smell is the chief or only factor to be considered, the most reasonable theory is to suppose that plants, like animals, emit odors, and that insects in searching for food, either for themselves or their progeny, are guided by them. It may also be assumed that the volatile chemical constituents of plants emit particular odors, and that the odor emitted from a plant is a combination of all the odors from these constituents, or possibly one odor may be so strong that it masks all the others. Upon this hypothesis, the odors from plants would vary according to the number, combination, and quantitative percentages of the various volatile constituents. Reasoning along this line, we may be able to explain why a few insects have only one host plant; why many have a preferred host plant, but will eat allied plants; and why others feed upon a large number of plants. If we had positive information on this subject, we might be able to devise practical methods for the control of the boll weevil and certain other insect pests by using attractive poisoned baits, or by placing the "attractant" in traps.

[1] Received for publication July 3, 1926; issued January, 1927.

The foregoing is only a brief theoretical statement of how plants attract insects by odors. At once the reader may rightly ask, "Is there any definite information to support this theory, and are insects really attracted by odors from a distance?" A mass of literature dealing indirectly with this subject has been published, but very little of it is actually applicable to this particular problem, chiefly because nearly all of the investigators have used certain chemicals, or other compounds, not necessarily plant constituents, and for that reason it is difficult to correlate the results. For lack of space only a few references will be cited. Verschaffelt's (*57*)[2] results are the most important along this particular line. He observed that cabbage butterflies seem to be attracted to various members of the Cruciferae, and found by tests that their larvae ate the leaves of 15 species, representing 14 genera of this family. Chemists had previously determined that all of these contain mustard oils; thereupon Verschaffelt devised experiments and proved that the larvae actually seek out various mustard oils.

There is no definite or reliable information to show that insects are attracted from long distances by plant odors, but members of the Biological Survey of this department have had success in attracting mountain lions, wildcats, and lynxes from a distance to traps baited with oil from the catnip plant, although this particular subject may have little or no bearing on that discussed in this paper. Nevertheless, we do have definite information about the attraction of insects from long distances by insect odors. Riley (*50, p. 38–39*) reports that a caged female of the Ailanthus silkworm moth of Japan was apparently scented from a long distance by a marked male of the same species, which had been carried one and a half miles one evening and then released; the next morning the two moths were together. Fabre (*8, p. 189–216*), Forel (*9, p. 76*), and Mayer (*40*) conducted many experiments to prove that male moths apparently by smell alone can easily locate their females from long distances.

No direct evidence has been presented to support the theory that cotton plants really attract boll weevils by means of odors or emanations, but Power and Chesnut (*44, 45*) have shown experimentally that living cotton plants do give off emanations containing two odorous substances, one of which has an extremely strong and persistent odor, and the present writer (*38*) in 1925 experimentally convinced himself that the Colorado potato beetle (*Leptinotarsa decemlineata* Say) is attracted partially, if not wholly, by the emanations from its host plants.

While conducting experiments at Tallulah, La., in July and August, 1922, to determine whether or not the practice of dusting cotton plants with calcium arsenate is injurious to honeybees, the writer was asked by B. R. Coad, in charge of the Delta Laboratory at Tallulah, to study the behavior of the cotton boll weevil in the field and laboratory. During the latter half of June, 1924, the writer was again at Tallulah, collecting more material and conducting further experiments on the boll weevil. The results obtained are herein recorded. Although only of a preliminary nature, they add considerable weight to the remainder of this report, which is of a morphological character.

[2] Reference is made by number (italic) to "Literature cited," p. 1139.

The writer has made a thorough study of the anatomy of the sense organs of the boll weevil, and has brought together the available information along this line in the hope that new light may be thrown on the entire subject.

ATTRACTIVENESS OF COTTON PLANTS TO BOLL WEEVILS

The boll weevil, compared with many other insects, is a weak flyer, but during the dispersal period in autumn it has been known to migrate more than 40 miles in a short time by successive short flights. At other periods it is comparatively quiet and seldom travels far, although in spring, when it emerges from hibernation, it may be a mile or more from the nearest cotton. It is, however, usually much nearer. Regardless of its distance from cotton plants, Hunter and Pierce (*18, p. 41*) inform us that it apparently makes no mistakes in finding its favorite food.

The cotton boll weevil is known to eat normally several species of plants; but when forced to feed upon others to which it is not accustomed, it will partake of them only to a limited extent; however, practically all the plants which it will eat belong to one family, the Malvaceae. Coad (*4, p. 3*) and Smith (*53, p. 5*) state that the boll weevil normally feeds on the various species of the cultivated and wild cottons (*Gossypium hirsutum, G. herbaceum, G. barbadense, G. brasiliense, G. davidsoni*, etc.) and also on the so-called wild cotton (*Thurberia thespesioides*). Coad succeeded in rearing weevils on the buds of the shrubby althaea (*Hibiscus syriacus*), but had only partial success in rearing them on the buds of *Callirrhoe involucrata, C. pedata*, and *Sphaeralcea lindheimeri*.

CHEMOTROPIC EXPERIMENTS

Hunter and Hinds (*17, p. 70–71*) report that owing to the belief that it might be possible to destroy the weevil by attracting it to sweetened poisons, a number of experiments were performed in the laboratory and field. Various grades of sugar, molasses, and honey were used in comparison with the attractive influence of cotton leaves. None of the sweets seemed to exert any noticeable attraction, except the honey, and that only slightly; but the cotton leaves always attracted many weevils. Hunter and Pierce (*18, p. 43*) corroborate these findings.

Recently this theory has been revived and many tests conducted to determine whether it has any practical application. McGehee (*26, p. 7, 15*) does not believe that the molasses in the sweetened poison attracts the weevils, although this method compared favorably in its results with the dust method. Leiby and Harris (*24, p. 9, 12, 18*), who tested a proprietary sweetened poison, and home-mixed ones containing blackstrap molasses, state that their findings are inconclusive as far as results are concerned. Isely and Baerg (*19, p. 16, 17, 23*) used proprietary sweetened poisons and found that they appeared less effective than the dust method and involved much more labor.

EXPERIMENTS IN WHICH SWEET SUBSTANCES, WATER, COTTON LEAVES, AND COTTON SQUARES WERE USED

In order to find a substance, preferably a sweet one, which would attract boll weevils equally as well as do cotton squares, the experiments described below were performed at Tallulah, La., during July

and August, 1922. If such a substance were found, it could be mixed with poisons and applied in cotton fields to control the weevils; or traps baited with it might be installed in the fields for the purpose of catching the weevils. The writer was ably assisted by G. L. Smith, who performed the field tests and aided with those at the laboratory.

Preliminary Experiments

The first few preliminary experiments showed that weevils pay little or no attention to three brands of molasses (blackstrap, koo-koo, and karo), honey, saccharine (strong solution), and sugar sirup (saturated solution).

Fifty weevils, collected the day before being tested, were put in each of eight wire-screen cages (12 inches square by 1.5 inches deep) which were placed side by side on supports in the shade of a large cottonwood tree. Experience quickly showed that most of the weevils went to one corner or to one side of the cage and usually remained there, regardless of the substance placed in the cage; therefore, in order to force them to change their position, it was necessary before testing them to reverse the cages end for end. The seven liquids used were put on small pieces of absorbent cotton of equal size, and the pieces of cotton were then laid on small pieces of paper of equal size, one of which was placed at the center of each of seven cages. A cotton square, lying on a piece of paper of the same size, was also placed in the center of the eighth cage. Counts were made every minute up to 10 minutes, when the liquids and cotton squares were shifted from cage to cage, so that at the conclusion of the experiments each of the eight cages had contained each of the eight substances. A summary of these eight series of tests at the end of the 10 minutes showed the total number of weevils which had eaten of the substances to be as follows: Cotton square, 27; water, 8; honey, karo, and koo-koo, 7 each; sugar sirup and saccharine, 5 each; and blackstrap, 3.

The following morning about half of the weevils were dead. The foregoing experiments were repeated by using 25 of the remaining weevils, now hungry, in each cage. A summary of these tests at the end of 10 minutes showed the number of weevils which had eaten of the various substances to be as follows: Cotton square, 21; water, 11; honey and karo, 6 each; sugar sirup and koo-koo, 3 each; blackstrap, 1; and saccharine, 0.

The preceding experiments were repeated three times, 50 fresh weevils being used in each cage each time, although in these tests the liquids were dropped upon fresh cotton leaves which were placed at the centers of the cages. A summary of all three sets gives the following results: Cotton square, 172; water and sugar sirup, 58 each; karo, 53; honey, 44; koo-koo, 35; blackstrap, 21; and saccharine, 15.

Tests in which 30-minute periods were used instead of 10-minute ones did not materially change the above proportions.

Tests in Field Cages

Experiments somewhat similar to the preceding, but performed on a larger scale, were carried on by releasing 100 fresh weevils in each of 7 field cages, 4 feet square by 4 feet deep. The cages were turned upside down and heavy muslin used as tops, which made more or less shade for the weevils. The various liquids to be tested

were dropped upon fresh cotton leaves, one of which was then placed at the center of each side and bottom of the cage. Cotton squares and leaves not bearing any liquid were similarly placed. The weevils were released at 10.30 a. m., and counts were made every half hour up to 5 p. m. A summary of two sets of experiments, showing the maximum number of weevils at one time on all the leaves or squares in a cage, is as follows: Cotton square, 28; koo-koo, 7; honey, 6; blackstrap, 5; karo, 4; leaves (control), 4; and sugar sirup, 1.

The field cages used in the preceding experiments were moved to a cotton field and each was placed over a cotton plant in the usual manner when such cages are employed. At 8.30 a. m., about 12 leaves at the top of a plant were treated with drops of either sugar sirup, honey, karo, koo-koo, blackstrap, or with ice-cream powder in a liquid form. The control plant was left untreated. At 9 o'clock, 100 fresh weevils were released in each cage, and thereafter up to 5 o'clock the number of weevils found on the treated plants and control were counted hourly. This set of weevils was used two days, the liquids being applied to the leaves again on the second day.

These experiments were repeated twice, fresh weevils being used each time. The second set of insects was used three days and the third set two days. In these five tests a sweetened proprietary calcium arsenate mixed with water was also applied to the leaves in addition to the substances enumerated above.

TABLE 1.—*Maximum number of boll weevils found at one time on untreated plant and on other cotton plants variously treated*

Test No.	Number of weevils on untreated plant	Number of weevils on plants treated with—													
		Sugar sirup		Honey		Karo		Koo-koo		Blackstrap		Ice-cream powder		Sweetened proprietary calcium arsenate	
		Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves	Entire plant	Treated leaves
1	89	91	3	92	4	92	1	86	1	79	1	88	5	------	------
2	84	83	5	85	7	80	2	81	2	66	1	86	5	------	------
3	85	88	3	85	3	91	2	72	2	60	0	85	4	62	0
4	96	94	3	82	3	95	2	82	3	96	0	95	4	90	0
5	83	78	4	73	2	85	3	69	3	74	2	93	3	63	0
6	91	86	3	91	3	95	3	88	1	85	2	95	3	95	0
7	70	84	3	73	2	85	1	82	2	73	0	85	2	86	0
Average	85	86	3	83	3	89	2	80	2	76	1	90	4	79	0

Tables 1 and 2 show the detailed results of these seven tests. It is seen that the plants treated with karo and ice-cream powder bore a few more weevils than did the control; those treated with koo-koo, blackstrap, and sweetened calcium arsenate bore a few less; and those treated with sugar sirup and honey bore practically the same number as did the control (Table 1). Since these differences are

so small, they may be attributed to probable errors. The maximum number of weevils found at one time on the treated leaves shows that the liquids used had little or no attractiveness, but the blackstrap and sweetened calcium arsenate seem to have been slightly repellent (Table 1). Relative to the total number of weevils found on the treated leaves during the full period of the experiments, Table 2 shows that honey and ice-cream powder might have had a slight attractiveness, although so slight that they could have been of no practical importance.

TABLE 2.—*Number of boll weevils found on treated leaves of cotton plants during the full period of experiments*

Test No.	Number of weevils on leaves treated with—						
	Sugar sirup	Honey	Karo	Koo-koo	Black-strap	Ice-cream powder	Sweetened proprietary calcium arsenate
1	7	15	2	2	2	13	
2	11	15	5	4	1	13	
3	11	18	8	6	0	13	0
4	6	12	3	6	0	7	0
5	12	10	6	8	4	18	0
6	11	7	8	1	6	15	0
7	8	6	3	5	0	11	0
Average	9	12	5	5	2	13	0

CONCLUSIONS

The results obtained in this investigation indicate that the various substances used attract boll weevils very little or not at all, and none of them is of any practical value as a means of controlling these insects.

OLFACTORY EXPERIMENTS

The following observations were made by the writer in 1922 while trying to find a sweet substance that would attract boll weevils. As already stated, nearly all of the weevils tested in the cages (12 inches square) for some unknown reason went to the west side, and there remained regardless of the substances in the cages. Just as soon as the cages were reversed end for end, the weevils immediately walked from the east to the west side, and in so doing many of them passed within one-quarter, one-half, or three-quarters of an inch of the sweet substance on the absorbent cotton or on the cotton leaves, yet in hardly an instance did they turn from their straight course to go to it. They behaved differently, however, toward the cotton squares. Many of them went directly to the squares from a distance of 4 or 5 inches; and often a weevil turned at right angles while passing a square in order to reach it. If the honey and five brands of sirup used had acted as attractants, the weevils certainly would have behaved similarly toward them; although in regard to all of these responses, sight instead of smell might have been the sense used.

In collaboration with this bureau, F. B. Power and V. K. Chesnut, of the Bureau of Chemistry, undertook to determine the volatile constitutents of the cotton plant in order that these substances might be used to attract boll weevils. This work was begun at Tallulah,

La., in the summer of 1923 by distilling large quantities of cotton plants, and owing to the complex nature of the investigation, it lasted for some time. All of the results are now published, including two papers (*44*, *45*) concerning the volatile substances. These writers believe that the alkalinity of the dew of the cotton plant is to be attributed, at least in part, to the presence of ammonia and trimethylamine, because these substances were found to be emanations from living cotton plants and were also identified in the dew collected from the foliage. Among the 12 individual substances found by them in the concentrated distillate, which included all the odorous and volatile constituents in the cotton plant, were 3 of particular interest for chemotropic tests. The so-called "essential" oil was about 0.003 per cent of the material employed. It had a strong, rather agreeable, and persistent odor. The ammonia and trimethylamine were present in appreciable quantities, but the ammonia largely predominated. So small an amount of trimethylamine as 0.0000005 gm. can be detected by its odor. In regard to the essential oil, ammonia, and trimethylamine, Power and Chesnut believe that only the trimethylamine serves as an attractant to allure boll weevils to cotton plants.

During the latter half of June, 1924, the writer used for the first time a sample of the concentrated and strongly odoriferous trimethylamine (33 per cent in water), which was isolated from cotton plants by Power and Chesnut. Several simple tests were made similar to those in which the sweet substances mentioned above were employed, but in no instance was any perceptible attraction observed. The next step was to develop a special apparatus to test this chemical further. A crude device, now greatly improved, and called by the writer an insect "olfactometer," was made. A brief summary of the results obtained in 1925 by the use of this instrument with other insects is given on pages 1135–1136. During June of 1924 only a comparatively few old weevils, which had come to the cotton fields from their hibernating places, could be collected daily, and it was therefore necessary to use the same weevils repeatedly until they failed to respond. Being thus handicapped by lack of material and with only a crude apparatus, the writer could not obtain accurate and positive results, but the preliminary laboratory experiments showed that when the trimethylamine was sufficiently diluted with water the weevils seemed to be slightly attracted.

Some of the old weevils mentioned above were put singly in small wire-screen cages with glass tops, the inside measurements of the cages being 5 inches long, 2 inches wide, and ½-inch deep. All the chemicals tested in these olfactory experiments were held in vials of equal dimensions and capacity. When a weevil was ready for testing, the stopper of one of the vials was removed and the vial was placed just beneath the weevil on the bottom of the cage. In each instance the source of the odor was about three-fourths inch from the weevil. In nearly all cases only the reaction times of the first responses observed have been recorded, the time being registered with a stop watch the seconds of which were divided into fifths.

Trimethylamine (1 to 100): 3 turned to one side quickly; 2 turned around over vial and flew away; 2 turned around over vial and walked away; 1 moved quickly and then flew; 1 moved slightly, then tried to get through wire screen over vial; 1 moved quickly, then tried to put snout through wire screen, and rubbed hind legs together; reaction time 2 to 10 seconds, average 3.9 seconds.

Trimethylamine (1 to 1,000): 4 moved away slowly; 2 turned around and rubbed legs together; 1 raised wings; 1 moved an inch toward vial and turned around over it; 1 moving weevil stopped 5 seconds after vial had been held under it, then tried to put its snout through wire screen over vial; 1 turned around over vial and tried to put snout through wire screen; reaction time 3 to 7 seconds, average 4.9 seconds.

Benzyl acetate: 5 moved away quickly; 2 turned around quickly; 2 jumped quickly; 1 moved away and worked legs. Reaction time 1 to 4.4 seconds, average 2.6 seconds.

n-Butyl acetate: 7 moved away quickly; 2 moved away slowly; 1 jumped quickly. Reaction time 0.6 to 5 seconds, average 1.9 seconds.

Methyl acetate: 4 moved away slowly; 4 moved away quickly; 1 worked legs; 1 moving weevil stopped, then walked away. Reaction time 2 to 6.4 seconds, average 3.5 seconds.

Allyl alcohol: 5 weevils tested; reaction time 4 to 10 seconds, average 6.2 seconds.

Capryl alcohol: 5 weevils tested; reaction time 2 to 7 seconds, average 4.3 seconds.

One weevil was tested with each of the following: Anisole, n-butyl alcohol, caproic acid, caprylic acid, carvacrol, citral, p-cymene, iso-amyl alcohol, iso-amyl benzoate, iso-amyl formate, iso-butyl acetate, iso-butyl alcohol, iso-propyl acetate, iso-propyl alcohol, methyl ethyl ketone, n-propyl acetate, n-propyl alcohol, and tertiary amyl alcohol, but no reaction was shown which indicated that the weevil was really attracted by the odor emitted.

Conclusion

Not one in the list of 24 chemicals tested, except the trimethylamine, seemed to have any attractive properties. The trimethylamine appeared to attract weevils slightly at times, but there was never any pronounced attraction. During these tests the vial was placed beneath a few moving weevils to determine what effect the odor would have on their movements. In most cases the insect stopped, very often rubbed its legs together, then feigned death, but several seconds after the vial had been taken away, it "awoke" and moved on.

Lutz (*25, p. 275*) tested a large number of chemicals found either in flowers or employed in the manufacture of perfumes. He says:

> These were used with lard as a base in various concentrations and combinations. They were exposed in small dishes placed where flower-visiting insects were abundant, but no such visitors came to the dishes, although other insects (chiefly various flies) did so either by accident or otherwise. Possibly the right odors or combinations of odors were not used; possibly flower-visitors were not to be fooled by odors coming from such unflowerlike contraptions as glass dishes; and possibly insects do not find flowers by the sense of smell. Certainly, although the experiments consumed considerable time, they proved nothing.

SENSE ORGANS OF ADULT BOLL WEEVILS

Before entering into a discussion of the morphology of the sense organs of the boll weevil, it seems expedient to describe the material, methods, and histological technique employed in the study.

MATERIAL AND METHODS OF PREPARING WHOLE MOUNTS

The writer is often asked about his methods of preparing whole mounts of insects and about his histological technique, and since he has not published any details on these subjects in the past 10 years, he considers it worth while to give his most recent and best methods on these subjects, particularly since some of them have never been published.

Some of the material on the boll weevil was collected by the writer at Tallulah, La., in July and August, 1922; some in June, 1924; and other material was collected and preserved by various assistants of B. R. Coad, particularly by A. J. Chapman.

To obtain material for whole mounts of adult boll weevils, both dried and alcoholic specimens were used; but for whole mounts of larvae, only alcoholic specimens were employed. The specimens were treated with saturated caustic potash in the usual way by heating in a test tube over a Bunsen burner, or were allowed to stand in the unheated liquid for a day or longer, depending on their size and hardness. They were then removed and thoroughly washed with water, and the adult specimens were decolorized or bleached with chlorine gas in the following manner: A small quantity of potassium chlorate or chloride was put in a small wide-mouthed bottle (bleaching bottle). The specimens were then wrapped loosely in a small piece of cheesecloth which was suspended from a hook in the middle of the stopper. A pipetteful of hydrochloric acid was next dropped upon the potassium salt in the bottom of the bottle. At once chlorine gas was liberated, which in a few minutes bleached the dark-colored specimens. The specimens were again thoroughly washed in water and finally preserved in 50 per cent alcohol.

To study the external parts of the sense organs, 10 individuals of each sex and several larvae were dismembered by having all the appendages, head, thorax, and abdomen carefully severed. These parts were then put in vials in such a way that their identity was preserved. Some of these parts were temporarily mounted in 50 per cent alcohol, and others were mounted in Canada balsam or in a 15 per cent solution of potassium acetate. This solution, as well as the 50 per cent alcohol, gave a good refractive index, so that the minutest pores or slits could be easily discerned, but the balsam was less satisfactory in this respect. All parts were mounted between cover glasses in order that both sides of the specimen could be carefully studied under a high-power lens. In the case of the potassium-acetate mounts one cover glass was considerably smaller than the other in order that it might be securely sealed with asphaltum.

MATERIAL AND HISTOLOGICAL TECHNIQUE

To obtain material for the study of the internal anatomy of the sense organs, full-grown larvae and adult weevils (just ready to emerge) were taken from the flower buds, called "squares." To insure a good supply of material, a large number of squares bearing larvae and pupae were collected in the fields, and these were kept in the laboratory and examined daily. Some of the material was put at once in alcohol to be used for whole mounts, but most of it was preserved in the Bouin and the modified Carnoy fixing fluids. The latter, consisting of equal parts of absolute alcohol, chloroform, and glacial acetic acid, with corrosive sublimate to excess, was kept in a glass-stoppered bottle so that it might not lose its fixing ability by absorbing moisture from the air, although it does deteriorate within a few weeks in spite of all precautions taken. When material was dropped into vials containing this fluid, the stoppers were left off no longer than was absolutely necessary. To assure good fixation of the internal tissues, the snout or beak, antennae, head, legs, wings, and thorax were severed, and immediately dropped into the fixing

fluids, the Carnoy fluid being mostly used. The material preserved in the Bouin fluid was left in it overnight, and then washed several times in 95 per cent alcohol, but the material preserved in the Carnoy fluid was removed when it sank to the bottom of the vial, and then thoroughly washed in 95 per cent alcohol. All material was kept in 85 per cent alcohol until sectioned.

From this stage on, the procedure is considerably different from the double or paraffin-celloidin method described by the writer in 1915 (*30*). The successive steps in its use are as follows: Remove excess mercury in tissues fixed in Carnoy fluid by leaving pieces of material overnight in 85 per cent alcohol containing tincture of iodine (wine color); put small pieces of material containing sense organs in clear 85 per cent alcohol to wash out the iodine; put material in 95 per cent alcohol containing eosin; place pink tissue in absolute alcohol and ether (half and half) containing eosin, for only a few minutes; put material in tiny vials containing thin celloidin and leave overnight; next morning put vials in melted paraffin in small beaker on hot plate, and allow ether to boil gently for a few minutes; remove vials from paraffin, take out pieces of material, one at a time, and be sure that each has considerable celloidin surrounding it; drop each piece in chloroform and leave it until celloidin has become firm; remove these pieces from chloroform, dry on blotting paper, trim celloidin, and put them in paraffin in Petri dish on hot plate for a few minutes; when all bubbles have left them, transfer to tiny paper cups in another Petri dish containing the best and hardest paraffin (58° or 60° C., M. P.), and leave them there for a few minutes. (NOTE.—Never allow paraffin to become more than a degree or two higher than its melting point, for excess heat makes the tissue brittle.) With a very sharp knife cut the ribbons 5 microns in thickness, and if paraffin fails to ribbon properly, warm the knife; mount ribbons, using a thick film of fresh Mayer's albumen and flatten them by pressing on them with fingers, but use no water or heat, for heat blisters celloidin; let slides dry on top of paraffin oven 48 hours; after that put them in 50 per cent alcohol and then in Ehrlich's or Delafield's haematoxylin stain (well ripened) for three or four minutes; leave them in water one hour or longer, then run them up through the alcohols (50, 70, 85, and 95 per cent plus eosin), leaving them in each five minutes; then put them in clear 95 per cent alcohol to wash out the excess eosin; next in Gage's carbol-xylene (1 part carbolic acid to 3 parts of xylene) for only a few seconds, for this liquid slowly dissolves paraffin; lay them flat on match sticks upon the table, then drop xylene upon the slides gently with a pipette; after paraffin has dissolved, gently incline slides to allow xylene to run off them, then add Canada balsam and cover glasses.

Amateurs who try to follow these directions will doubtless have trouble, chiefly because experience and an understanding of the principles involved mean much more to a successful microscopic technician than the mere ability to follow directions closely. For this reason the writer hesitates to give such a complicated method, knowing that someone will pronounce it of no value.

OLFACTORY ORGANS

Since the sense organs of the adult or imago weevils are the more numerous and diversified, they will be described first, and a discussion of the sense organs of the larvae will follow.

Owing to the belief that the sense of smell in boll weevils is the primary one which aids them in locating cotton plants, the writer has paid more attention to the olfactory organs than to the other sense organs.

Disposition of Olfactory Pores

Hicks in 1857 (*13*) first described the sense organs herein referred to as "olfactory pores," and suggested that they might serve as olfactory organs. Nagel (*42*) saw some of them on the mouth parts of *Dytiscus marginalis*, and called them "Gruben ohne Kegel." Hochreuther (*15*) found them on the same species, and called them "Kuppelförmige Organe." He saw a few on the epicranium; 11 on the scape and pedicel?; a few on the labrum; very few on the mandibles; 6 to 8 on the maxilla; 14 to 16 on the labium; and 10 to 20 on the coxa, trochanter, femur, and tibia. Lehr, in 1914 (*22*), described them on the wings and elytra of the same beetle. Before this date they had been seen in other insects, and were called "organes sensitifs à ombelle" by Janet (*20*), and "sensilli campaniformi" by Berlese (*1*). Years ago the writer (*28, 29, 30*) studied them thoroughly in Hymenoptera and Coleoptera, called them olfactory pores, and determined to his own satisfaction that they serve as olfactory organs.

Several years ago J. A. Nelson, of this bureau, began a thorough study of the morphology of the boll weevil, but resigned before he had accomplished much. However, he prepared a 3-page manuscript on the sense organs, which still remains unpublished. Most of his time he spent in studying the disposition of the olfactory pores, but he also noted the tactile hairs and hypodermal gland pores. He found the olfactory pores located in about the same positions as recorded by the present writer in the following pages, and he saw a few very minute pores, presumably olfactory ones, on the tibial spines. In this insect the present writer did not note these, but has described them in the tibial spines of many other beetles. Nelson says, in part:

> These pores are excessively minute, measuring 5–7 micra in diameter. Largely for this reason a study of their histological structure was found to be impossible. * * * The most satisfactory sections were those of the pores at the bases of the antennae (of pupae). As far as could be determined, these correspond in structure to the so-called olfactory pores described by McIndoo, but the nature of the material makes it decidedly unfavorable for a study of the minute structures of these organs. It should be noted finally that in general the distribution and appearance of all of the sensory pores mentioned accord closely with the account given by McIndoo (1915). * * * Since McIndoo has brought sound experimental evidence to show that the pores described by him are actually olfactory in function in a wide range of insects, including several representatives of the Coleoptera, it would seem reasonable to extend this view to cover *Anthonomus*.

The elytra, wings, maxillae, and labium have dorsal and ventral surfaces; the submentum, only a ventral side; and for descriptive purposes, the antennae, legs, and mandibles may be divided into outer and inner surfaces. Since female No. 3 was studied most critically and all the drawings were made from this specimen, the disposition of its olfactory pores will be described in detail, and then will follow a discussion of the individual and sexual variations found in these organs in five females and five males.

Group 1, consisting of 53 pores (fig. 1, A, B), lies on the dorsal surface of the elytron, being found on the radial plate (RP) between the muscle disk (MD) and the subcostal head (ScH), with its distal

or broader end against the basal margin (BM) of the elytron. Groups 2 to 6 (C) lie on the wing, Nos. 2 to 5 being on the dorsal surface and No. 6 on the ventral surface; No. 2, of 25 pores, No. 3, of 30 pores, and No. 4, of 104 pores, lie on the radius (R); No. 5, of 4 pores, on the first cubitus (1Cu); and No. 6, of 26 pores, on the media (M).

Groups 7 and 8 and isolated pores *a*, *b*, and *c* are found on the legs (fig. 2); single pore *a* lies on either surface at the proximal end of the femur; *b* and *c* at the proximal end of the tibia, *b*, consisting of two widely separated pores, lies on the outer surface, and *c*, of a single pore, on the inner surface. Nos. 7 and 8 lie, respectively, on the outer and inner surfaces of the trochanter; No. 7 on the front legs consists of 5 pores, but of 6 pores on the middle and hind legs; No. 8 on the front and middle legs consists of 2 pores, but of 4 pores on the hind legs.

Groups 9 to 11 and isolated pores *d* to *n* are found on the mouth parts (fig. 3); *d*, *e*, and *f* (each of 1 pore), *g* (2 pores), No. 9 (4 pores), and No. 10 (5 slits) lie on the ventral surface of the maxilla, *d* being found on the stipes, *e* on the palpifer, No. 9 on the first segment of the maxillary palpus, *f* on the second segment, and *g* and No. 10 on the third or distal segment of the same palpus (fig. 3, A and B). On the dorsal (partly lateral) surface of the maxilla are found the isolated pores *h*, *i*, and *j*; *h* (1 pore) lies on the palpifer, *i* (1 pore) on the first segment of the maxillary palpus, and *j* (2 pores) on the third segment of the same palpus (C). On the submentum lies *k* of 5 pores, and on the ventral surface of the labial palpus lie *l* and *m*, each of 1 pore, *l* being found on the first segment and *m* on the third segment (D). The dorsal surface of the labium is devoid of pores (E). No. 11 of 4 pores and *n* of 2 pores lie on the outer surface of the mandible; the inner surface of the mandibles of this specimen bore neither pores nor hairs (F and G).

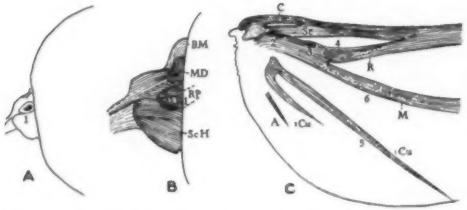

FIG. 1.—Portions of right elytron and right wing of female boll weevil No. 3, showing location of Groups 1 to 6 of olfactory pores as indicated by the numbers 1 to 6

A and B, peduncle of elytron, showing Group 1 on dorsal side of radial plate (RP); and C, showing Groups 2 to 5 on dorsal surface, and Group 6 on ventral surface of wing. A, × 19; B, × 38; and C, × 28

Abbreviations: A, anal vein; BM, basal margin; C, costal vein; 1Cu, first cubital vein; 2Cu, second cubital; M, media; MD, muscle disk; R, radius; Sc, subcostal vein; and ScH, subcostal head

On the head capsule lie 10 pairs of pores (fig. 4, A and B); 2 pairs (*o*) are found just behind the compound eye on the epicranium; 3 other pairs (*p*) on the epicranium; 4 pairs (*q*) on the occiput; and 1 pair (*r*) on the genae (B).

At the base of one antenna lie 4 pores (C); 2 (*s*) being found on the outer surface and 2 (*t*) on the inner surface of the scape; but the other antenna of this specimen bears only 3 pores (A and B).

In regard to the individual variations only a few remarks need be made because most of the minor differences observed may have been due to the writer's inability to find the pores, particularly where the chitin was dark. As a rule, most of the groups of pores were fairly

constant in position, and varied only in the number of pores they contained. Some of the minor groups were often absent, or sometimes were united so that two were counted as one. No. 1 on the peduncle of the elytron was always present, and never had less than 45 pores. The 5 groups on the wings were sometimes reduced to 4, but the total number of pores was thereby seldom decreased; No. 5 ranged from 0 to 6 pores; No. 6 from 26 to 67; and in one instance No. 6 extended entirely to the fold in the wing. The disposition of

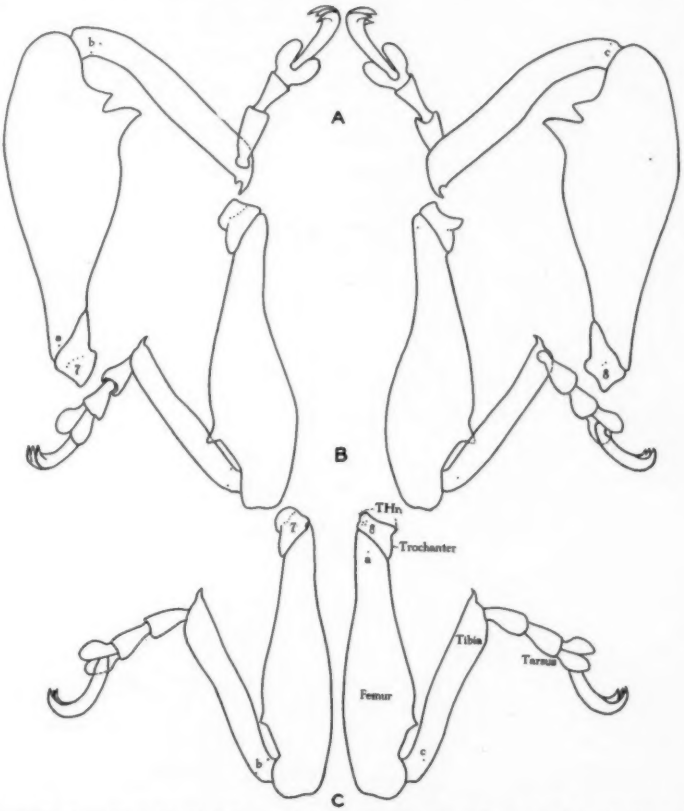

FIG. 2.—Portions of legs of female boll weevil No. 3, showing location of Groups 7 and 8, and isolated olfactory pores *a*, *b*, and *c*; tactile hairs (THr_1); A, front leg; B, middle leg; and C, hind leg. The drawings at the right represent the inner surface and those at the left, the outer surface. All × 24

the pores on the legs, mouth parts, and antennae was similar to that already described, and the only striking difference found was in the total number of pores. Group 10 (fig. 3, B) on the third or distal segment of the maxillary palpus was always present and in each case seemed to consist of 5 slits. Only one male and one female were examined for pores on the head capsule (fig. 4, A and B), and no variations were noted; therefore probably all the head capsules bear these organs.

Relative to these pores, no sexual variations were found other than what might be regarded as individual differences, except that the females always had a greater number of pores than the males. Table 3 shows the individual and sexual variations in the total number of pores found. The pores varied in part as follows: Legs: Females, 22 to 28; males, 19 to 25. Elytra: Females, 90 to 106; males, 90

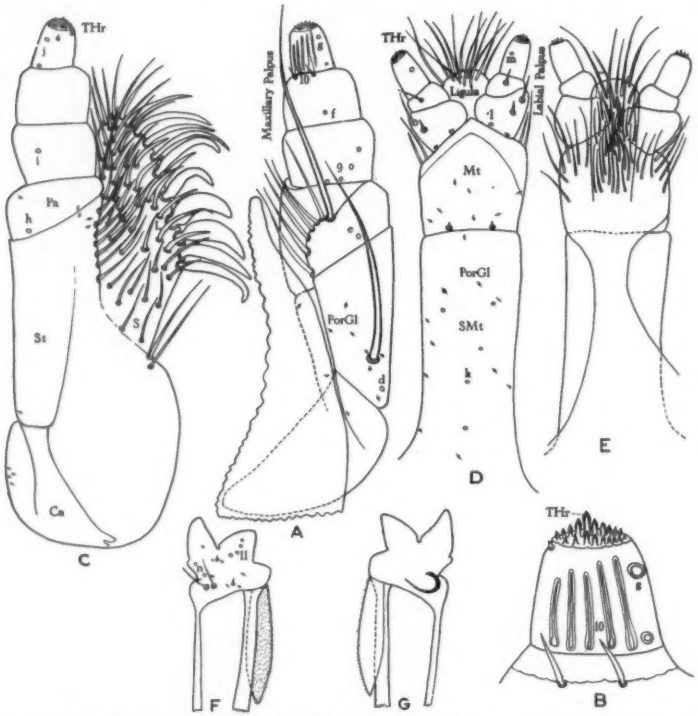

FIG. 3.—Portions of mouth parts of female boll weevil No. 3, showing location of Groups 9 to 11 and isolated olfactory pores *d* to *n*; tactile hairs (THr); and gland pores (PorGl). A, B, and C, maxilla; A, ventral surface; C, dorsal-lateral surface; and B, dorsal surface of distal or third segment of maxillary palpus. D and E, ventral and dorsal surfaces, respectively, of labium. F and G, outer and inner surfaces, respectively, of mandible. A, C, D, and E, × 200; B, × 522; and F and G, × 56

Circles and slits represent the olfactory pores, while black dots represent the gland pores, the lines through the latter showing the direction in which their efferent tubes extend. Ca, cardo; L, lacinia; Mt, mentum; Pa, palpifer; S, subgalea; SMt, submentum; and St, stipes

to 96. Wings: Females, 376 to 413; males, 322 to 376. Total number: Females, 604 to 641; males, 527 to 576. Average: Females, 624; males, 549. Thus the females have 13.7 per cent more pores than have the males. It will also be noted that about two-thirds of the pores are borne by the wings, and nearly five-sixths by the wings and elytra combined.

External Structures of Olfactory Pores

When examined under a low-power lens, the large olfactory pores may be easily mistaken for hair sockets from which the hairs have been removed; and the smaller ones may be mistaken for the pores of hypodermal glands, or vice versa. When they were more care-

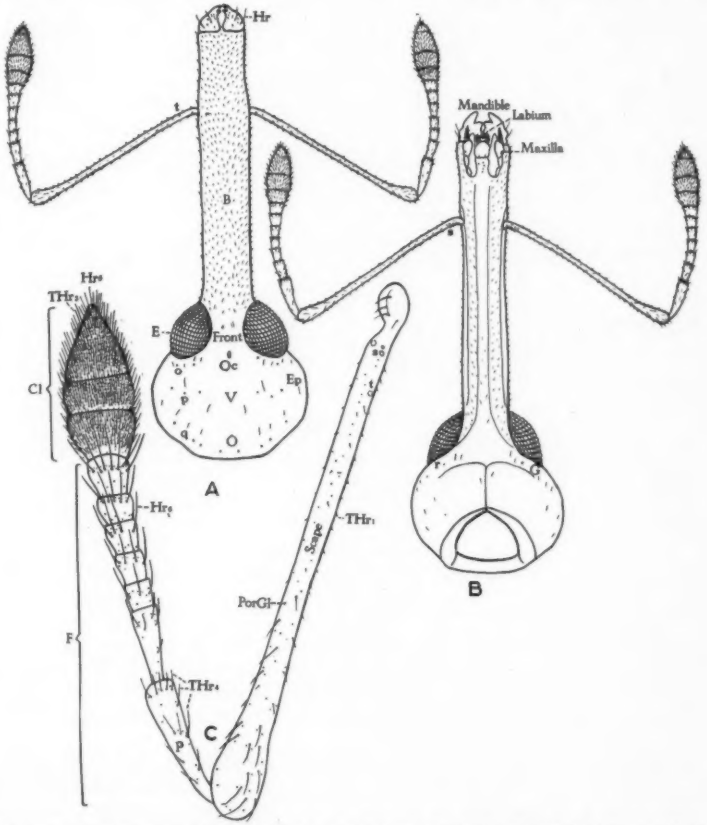

FIG. 4.—Head and its appendages of female boll weevil No. 4, showing location of olfactory pores (*e* to *l*), tactile or innervated hairs (THr_1, THr_3, THr_4), noninnervated hairs (Hr, Hr_5, and Hr_8), and gland pores (PorGl). The disposition of olfactory pores is tolerably accurate but that of the hairs and gland pores is only approximate. In A and B the olfactory pores are represented by dots, but in C by circles (the dotted ones lie on opposite side of scape). In A and B the gland pores are not shown, but they are very numerous on the beak or snout, moderately numerous on the antennae, rather scarce on the mouth parts, and few on the head capsule; in C they are approximately located by dots. A, dorsal view of head and inner surface of antenna, × 24; B, ventral view of head and outer surface of antenna, × 24; and C, outer surface of left antenna, × 60

Abbreviations: B, beak [or snout; Cl, club; E, compound eye; Ep, epicranium; F, funiculus; G, gena; O, occiput; Oc, ocellus; P, pedicel; and V, vertex

fully observed under a high-power lens, it was usually not difficult to distinguish them from the hair sockets and gland pores. Both types of pores and hair sockets usually appear as small, round, bright spots when a strong transmitted light is used, but after a more careful study all three structures were found to differ widely. The hair

sockets generally appear the least bright; their walls are usually heavier and darker; and their pore apertures are usually bounded by rough or torn chitin, thus showing the mutilation caused by removing the hairs. The gland pores (fig. 5, A and B) as a rule are much smaller than the olfactory pores (fig. 5, C to L); they are many times smaller than those on the legs; usually much smaller than those on the wings and antennae; but almost the same size as those on the elytra and mouth parts. It is often difficult to distinguish a hair socket from an olfactory pore, but a gland pore is always easily recognized by its aperture (A, PorApGl) which, when one focuses downward on it, is observed to run V shaped from the pore wall (PorWGl) to the center or entirely across the pore.

TABLE 3.—*Disposition of olfactory pores on adult cotton boll weevils*

Appendages examined	Females					Males				
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 1	No. 2	No. 3	No. 4	No. 5
Front legs	22	25	22	22	22	23	22	20	22	22
Middle legs	24	26	24	24	24	22	19	24	24	24
Hind legs	27	25	28	28	28	22	25	22	22	22
Elytra	106	92	106	90	94	90	96	90	94	91
Wings	376	413	378	411	400	322	345	376	328	340
Mandibles	12	8	12	8	8	6	10	4	7	8
Antennae	7	7	7	6	7	6	6	6	6	6
Maxillae	23	36	36	29	36	27	23	25	26	27
Labium	5	4	4	4	4	4	4	4	4	4
Submentum	2	5	5	4	5	5	5	5	5	4
Total	604	641	622	626	628	527	555	576	538	548
	Average for females, 624 [a]					Average for males, 549 [a]				

[a] These numbers should be increased to 644 and 569 to include the 20 pores found on the head capsule each of a female and male.

The external structure of an olfactory pore consists of the aperture, wall, and border. The aperture (fig. 5, G, PorAp) may be round, oblong, or slit shaped (H). The wall (C, PorW) may be round, oblong (E), eye shaped (G), or slit shaped (fig. 3, B, and fig. 5, H). The border (fig. 5, G, PorB) may be round or oblong, but it was not discernible in most cases; on the wings it was usually present and surrounded each pore wall, although on the elytra it surrounded the entire group (fig. 5, E).

Most of the olfactory pores are round or oblong (fig. 5, C to E), but a few are eye shaped (fig. 5, F and G), and a few others are slit shaped (fig. 3, B, and fig. 5, H). The round or oblong ones are found on the femora, tibiae, elytra, wings, mouth parts, head, and antennae; the eye-shaped ones, on the wings; and the slit-shaped ones, on the trochanters and third segments of the maxillary palpi. In external structure the slit-shaped ones resemble the slits in the lyriform organs described in spiders by the writer (*27*) and others. Not all of those on the trochanters are as slender as the one shown (fig. 5, H), but they vary from this shape to the extremely elongated type.

INTERNAL STRUCTURE OF OLFACTORY PORES

In sections the olfactory pores are never mistaken for the gland pores (fig. 5, M to O) because they are usually much larger, and, besides, the reservoirs (Re) of the gland pores stand out quite con-

spicuously in the chitin. From the reservoir the efferent tube (Ef) leading to the exterior is also plainly seen, but the large gland cell (GlC) is not always easily distinguished from the hypodermal cells (HypC), although whenever it is discernible a portion of it runs into the pore canal (PorC) leading to the reservoir, and a clear area or ampulla (Am) in the gland cell lies just beneath the pore canal. These glands are almost like the young stages of those described in the squash beetle (*Epilachna borealis* Fab.) by the writer (*31*).

The olfactory pores (fig. 5, P to D′) are more or less flask-shaped structures, and all really belong to one type, for as a rule their apertures open to the exterior on the same level as the surrounding chitin, and not into pits or at the tops of small domes as described by the writer (*30*) for some other beetles. However, the one represented in Figure 5, P, approaches the pit-shaped type, and the one in Figure

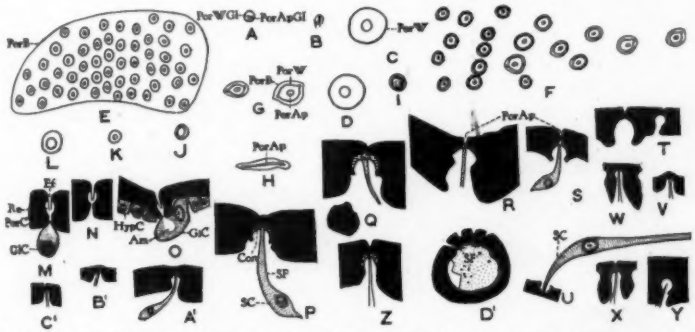

FIG. 5.—Cotton boll weevil: External structure of gland pores (A and B) and olfactory pores (C to L). A, from submentum; B, from antenna; C, from tibia of middle leg; D, from femur of middle leg; E, Group 1 on peduncle of left elytron; F, portion of Group 4 on wing; G, 2 pores from distal end of Group 4; H, one of slit-shaped pores on trochanter of hind leg; I, from first segment of maxillary palpus; J, from distal segment of labial palpus; K, from submentum; and L, from antenna. Internal structure of gland pores (M to O) and olfactory pores (P to D′). M and N, from antennae; M being from a section and N from a caustic-potash preparation, showing that the efferent tube (Ef) is chitinous. O, from ventral side of abdomen; P, from tibia; Q, from femur; R, from trochanter; S and T, from antennae, S being from a section and T from a caustic-potash preparation; U, from Group 4 on wing; V, from Group 6 on wing; W, from Group 2 on wing; X, from Group 1 on peduncle of elytron; Y, from head capsule (cut obliquely); Z, from mandible; A′, from labial palpus; B′, from stipes of maxilla; C′, from second segment of maxillary palpus; and D′, cross-section of third or distal segment of maxillary palpus

Abbreviations: PorAp, aperture of olfactory pore; PorApGl, aperture of gland pore; PorB, border of olfactory pore; PorW, wall of olfactory pore; and PorWGl, wall of gland pore. Am, ampulla; Con, chitinous cone; GlC, gland cell; HypC, hypodermal cell; PorC, pore canal; Re, reservoir; SC, sense cell; SF, sense fiber. All × 486

5, V, the dome-shaped type. The chitinous cones (Con) were usually visible, but the sense cells (SC) were not always found. Owing to the difficulty of obtaining good sections and to the minuteness of these organs, it was impossible to find one in which all the parts were present. Figure 5, P, shows the sense fiber (SF) piercing the cone, while Figure 5, R and S, and others show the pore aperture (PorAp).

The third or distal segments of the maxillary palpi were repeatedly sectioned in order to arrive at an understanding of the internal structure of their five slit-shaped organs, but after all of these attempts it was not definitely learned how the sense fibers unite with the apertures. The difficulties encountered will be appreciated when it is understood that the diameter of this segment was the same

as that of a hair from the writer's wrist, and it was impossible to secure good fixation of its tissues. The best cross section made is represented by Figure 5, D′, which shows that the knife passed through four of the five slits and that the aperture of one of these connects with the interior of the segment. It will be noted that one of the dark-staining bodies, the sense fibers (SF), unites with this aperture. Most of the sense cells present in the maxillary palpus lie in the first segment (fig. 6, A), while the remainder were seen in the second, but none was ever noticed in the third or distal segment. In the labial palpus (fig. 6, B) the sense cells are similarly located.

Figure 6, C, shows the large nerve (N) passing through the wing to group 4 on the dorsal surface. Attention is called to the sense cells (SC), trachea (Tr), thick and rigid chitin on the dorsal surface, but thin and flexible chitin on the ventral side. Figure 6, D, shows group 2 and other internal structures in a cross section of the wing.

Antennal Organs

It will be noted that the above heading falls under "Olfactory organs." The antennal structures are described in connection with the olfactory organs because it is commonly believed that the antennae

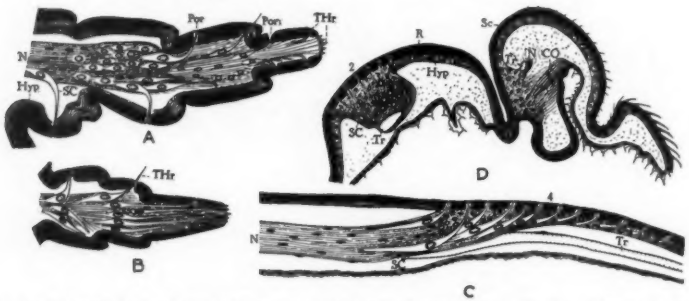

Fig. 6.—Cotton boll weevil: Semidiagrams, showing internal anatomy of maxillary palpus (A), labial palpus (B), and wings (C and D). A to C, longitudinal section; and D, cross section. C passes through Group 4, and D through Group 2

Abbreviations: CO, chordotonal organ ?; Hyp, hypodermis; N, nerve; Por, olfactory pore; Por_1, slit-shaped olfactory pore; R, radius; SC, sense cell; Sc, subcosta; THr, tactile hair; and Tr, trachea. A, C, and D, × 296; B, × 494

bear the organs of smell. The present writer, however, shows that the antennae do not bear organs suitable for this purpose, except the few olfactory pores at the base of each antenna. The antenna of the boll weevil is made up of three parts. The scape or scapus (fig. 4, C) consists of the first segment; the funicle or funiculus (F), of the second to eighth segments, but the second segment is also called the pedicel or pedicellus (P); and the club (Cl) consists of the ninth to twelfth segments.

The external anatomy of all the structures on the antennae of five males and five females was critically studied. Only three types of organs were found: (1) The 3 or 4 olfactory pores (Table 3) on the base of each scape (fig. 4, C), already described; (2) the gland pores, represented by dots widely distributed over the antenna; and (3) numerous hairs of various sizes. For descriptive purposes, the hairs are numbered from 1 to 6; but for comparative purposes, Nos.

1 to 4 are called sense hairs and sense bristles (Table 4). They are only approximately represented in Figure 4, C, and Figure 8, and in Table 4. The smallest ones, Nos. 1 and 2 (THr_1 and THr_2, fig. 7, A and B), were found almost entirely on the scape and club; the next in size, Nos. 3 and 4 (THr_3 THr_4, fig. 7, B and C), on all the segments, but mostly on the club; and the largest ones, Nos. 5 and 6 (Hr_5 and Hr_6, fig. 7, B and D), on the funicle and club.

The internal anatomy (fig. 8; fig. 9, A–G) of these hairs shows that all are innervated, except the largest ones, Nos. 5 and 6 (fig. 9, E and F). Numbers 2, 3, and 4 are most numerous, and those in the club seem to have a thread or extra fiber (fig. 9, C and D) running lengthwise in their sense cells from the base of the hair to the nucleus, or even beyond the nucleus. Figure 8 shows that the antenna is well supplied with nerves (N) and tracheae (Tr), but none of the innervated structures seems adapted to receive odor stimuli.

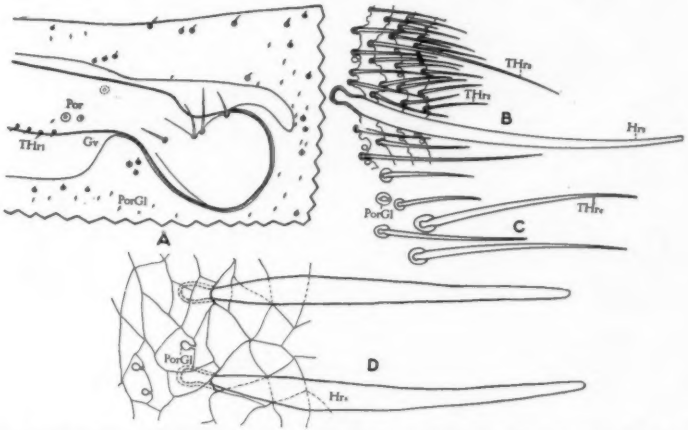

Fig. 7 (cf. fig. 4, C and fig. 8).—Cotton boll weevil: External structure of hairs on antenna and on beak at base of antenna. A, base of left scape lying in the antennal groove (Gv), showing tactile hairs, gland pores, and olfactory pores (the dotted one on opposite side); and B to D, besides showing hairs, show the antennal markings and gland pores

Abbreviations: Hr_5 and Hr_6, noninnervated hairs; Por, olfactory pore; PorGl, gland pore; and THr_1 to THr_4, tactile hairs. A, × 196; B to D, × 510

No experiments were conducted to determine whether the antennae of boll weevils carry the olfactory organs, but the writer in the past 10 years has tested many other insects having these appendages either cut off or otherwise mutilated. Since he has been criticized for using strong-smelling substances, chiefly essential oils, in order to induce the mutilated insects to respond to odors, he decided to conduct some experiments in which only the natural food odors would be used. Accordingly, two sets of potato beetles, each set of 50 individuals as usual, were tested in the insect "olfactometer" (*38*) by subjecting them to the odors from water extract, distillate, and emanations from living potato plants. The antennae were then cut off, and a few days later, when the insects had largely recovered from the operation, they were again tested daily with the odors and emanations. In all, 10 experiments, including 40 individual tests, were conducted. In all of the experiments except one the beetles

responded attractively to the plant odors, although slightly less so than before they were mutilated. Judging from these results alone and not considering the fact that the beetles had not totally recovered from the operation, we should say that the antennae bear only a few

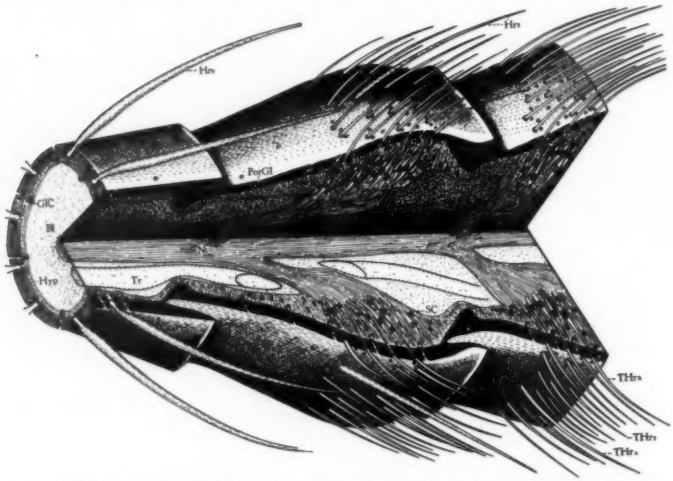

FIG. 8 (cf. fig. 4, C).—Cotton boll weevil: A schematic drawing of the ninth antennal segment with portions of the eighth and tenth, showing the antennal structures in perspective and in section. Note the following: Gland pores (PorGl), noninnervated hairs (Hr_5 and Hr_6), innervated or sense hairs (THr_2 to THr_4), none of which is suited for receiving olfactory stimuli. Bl, blood; GlC, gland cells; Hyp, hypodermis; N, nerve; SC, sense cell; and Tr, trachea

of the olfactory organs, which is contrary to the general opinion held about the location of these organs. Nevertheless, Minnich (*41*), who conducted many experiments with cabbage butterflies, partly

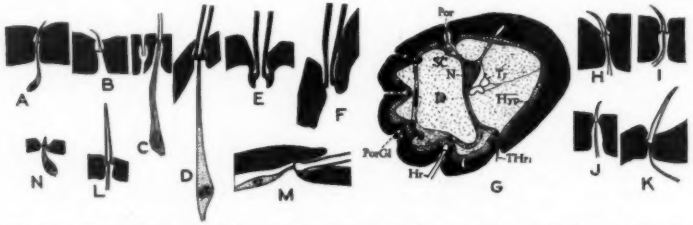

FIG. 9.—Internal structure of hairs of cotton boll weevil: A to D, innervated hairs, and E to F, noninnervated hairs on antennae. A is from a section, and B from a caustic-potash preparation, representing No. 1; C represents Nos. 2 and 3, and D, No. 4. Note the extra fiber in these two last sense cells. These fibers were only occasionally seen, but were not expected, considering the stain used. E represents No. 5, and F, No. 6. G, a cross section (semidiagrammatic) of base of scape passing through an olfactory pore (Por), a sense hair (THr_1), a noninnervated hair (Hr), gland pores (PorGl), and other structures; Bl, blood; Hyp, hypodermis; N, nerve; SC, sense cell; and Tr, trachea. H to N, tactile hairs from other parts of the boll weevil: H, from beak; I, from mandible; J, from head capsule; K, from thorax; L, from wing; M, from abdomen; and N, from ovipositor. A to F and H to N, × 490; G, × 314

supports the view of the present writer. He found that the amputation of both antennae of these insects reduced the olfactory response 58 per cent, and, accordingly, claims that the antennae do not constitute the sole olfactory area.

TABLE 4.—*Disposition of sense organs on head of adult cotton boll weevil*

Head and its appendages [1]	Sense hairs, Nos. 1 and 2 (Sinneshaare)	Sense bristles, Nos. 3 and 4 (Sinnesborsten)	Sense pegs (Sinneszapfen)	Pit pegs (Grubenkegel)	Olfactory pores (Kuppelförmige Organe)	Eyes	Johnston organ?	Chordotonal organ
Head capsule	Numerous on beak, especially near mouth parts and base of antenna.	Few on epicranium, vertex, front, occiput, genae.	Few near base of antenna.	None	10 on epicranium, 8 on occiput, 2 on genae.	2 compound eyes, 1 rudimentary ocellus.		
Antenna	About 51 on scape, 2 on funiculus, 280 on club.	About 12 on scape, 20 on funiculus, 200 on club.	None	do	3 or 4 on base of scape		In pedicel.	In pedicel.
Mandible	2 on outer surface	None	do	do	4 to 12			
Maxilla	2 on second segment of palpus.	Probably many on palpifer and lacinia.	19 at tip of palpus	2 near tip of palpus.	1 on stipes, 2 on palpifer, 15 on palpus.			
Labium	2 on mentum, 3 on palpus.	Probably many on mentum and ligula.	9 at tip of palpus	None	5 on submentum, 2 on palpus.			

[1] The labrum is absent in the boll weevil.

TACTILE ORGANS

Besides the tactile hairs (Nos. 1 to 4) already described on the antennae (fig. 9, A to G), innervated hairs were also found elsewhere; particularly on the mouth parts, other appendages, and even widely distributed over the body. The only safe way to determine a sense hair is to find its nerve in sections, but this is a difficult and slow task, and one can hardly be expected to do this for every individual hair; so after a careful study of the internal anatomy of certain groups had been made, the others were judged merely from their external structure. The internal anatomy of only a part of those recorded in Tables 4 to 6 were studied in sections, but since other investigators have found tactile hairs similarly located in other beetles, the writer feels that most of those which he calls sense hairs are really innervated, although perhaps he has overlooked certain other ones.

DISPOSITION OF TACTILE HAIRS

Hochreuther (*15*) made a thorough study of the sense hairs on a water beetle (*Dytiscus marginalis* L.). On the basis of external structure, he separated them into five divisions, one of which will be discussed under another heading, Since the writer knows of no similar work pertaining to a weevil, he has used Hochreuther's classification as a guide in the present investigation.

TABLE 5.—*Disposition of sense organ on thorax of adult cotton boll weevil*

Divisions of thorax and its appendages	Sense hairs	Sense bristles	Sense pegs	Pit pegs	Olfactory pores	Chordotonal organ
Prothorax	None	Several on margins of pronotum and poststernum.	None	None	None	
Mesothorax	Numerous on anterior margins of episternum and mesosternum; on prescutum and scutellum.	Same as for sense hairs.	do	do	do	
Metathorax	Several on anterior margin of metatergum.	Several on metatergum, metapleurum, and metasternum.	do	do	do	
First pair of legs	Several on coxa; 2 groups on trochanter; several at distal end of femur and on its teeth; few on tarsus.	Several on coxa; several widely distributed on femur, tibia, and tarsus.	do	do	6 to 8 on trochanter; 1 on femur; 3 on tarsus.	
Second pair of legs	Several on coxa; 2 groups on trochanter; remainder, same as above, but hairs less numerous.	Distribution same as on first pair of legs, but less numerous.	do	do	6 to 9 on trochanter; 1 on femur; 3 on tarsus.	
Third pair of legs	Same as above	Same as on second pair of legs.	do	do	7 to 10 on trochanter; 1 on femur; 3 on tarsus.	
Elytra	None?	None	do	do	45 to 53 on each peduncle.	
Wings	Few	Few	do	do	322 to 413 on both wings.	In base?

SENSE HAIRS NOS. 1 AND 2 (SINNESHAARE, or SENSILLA TRICHODEA).—Hochreuther found these on the head capsule, antennae, all mouth parts, thorax, legs, abdomen, and sexual apparatus. The present writer found them on the head and all its appendages (Table 4), parts of thorax, legs, and wings (Table 5), and on certain parts of the abdomen (Table 6).

SENSE BRISTLES, NOS. 3 AND 4 (SINNESBORSTEN, or SENSILLA CHAETICA).—Hochreuther found these on the epicranium, antennae, labrum, maxillae, labium, epipharnyx, cervical sclerites, thorax, legs, and abdomen. The present writer found them on the head capsule, antennae, and probably on the following: Maxillae, labium, thorax, legs, wings, and abdomen (Tables 4 to 6).

TABLE 6.—*Disposition of sense organs on abdomen of adult cotton boll weevil*

Divisions	Sense hairs	Sense bristles	Sense pegs	Pit pegs
Tergites	Few on each	Few on each	None	None.
Pygidium, or last tergite.	Few	Few	Few	Do.
Female genitalia	None	Several on palpus of ovipositor.	Few on palpus of ovipositor.	Many on ovipositor.
Male genitalia	None	None	None	Very numerous on body (or penis).
Sternites	Very numerous on last sternite; less numerous on other sternites.	Same as for sense hairs.	do	None.
Epipleurites	Few on each, near spiracle.	None	do	Do.

SENSE PEGS (SINNESZAPFEN, or SENSILLA BASICONICA).—Hochreuther found these on the head and all its appendages, thorax, legs, abdomen, and sexual apparatus. The present writer found probably a few on the head, maxillae, labium, and female genitalia.

PIT PEGS (GRUBENKEGEL, or SENSILLA COELOCONICA).—Hochreuther found these on the antennae, all mouth parts, epipharynx, mesothorax, legs, and sexual apparatus. The present writer found two near the tip of the maxillary palpus, and many on the female and male genitalia.

STRUCTURE OF TACTILE HAIRS

Vom Rath (*47*, *48*) found sense cells connected with all the small hairs on the maxillary palpi of *Coccinella septempunctata*, *Melolontha vulgaris*, and *Tenebrio molitor*, and also with all the small hairs on the labial palpi of the last species, in much the same manner as shown in Figure 6, A and B. Using Ehrlich's "Methylenblaumethod" and Golgi's "Chromsilberverfahren," Vom Rath traced the sense fibers of insects, myriapods, spiders, and crustaceans. In all the innervated hairs, whether short or long, and even in those at the tips of the maxillary and labial palpi, he saw a fine thread running lengthwise through each sense fiber to the tip of the hair, as shown in Figure 9, C and D, although the present writer was able to trace the sense fibers only to the base of the hair and never far into its cavity. Relative to Vom Rath's work, Hilton (*14*, *p. 566*), who has reviewed the literature on this particular subject, says:

> Earlier workers with methylen blue who studied simply the surface views represent nerves coming from the tips of the hairs; but it seems probable that

such figures are in large part diagrammatic. Vom Rath found by the Golgi method cavities of sensory hairs filled with nerves; this result is regarded as an artifact by Duboscq, who shows clearly how appearances like nerves may be obtained in the cavity of hairs due to deposits of chromate of silver; and he shows quite clearly that when nothing but the nerve cell and fiber is impregnated the nerve fiber stops at the base of the hair, as was apparently the case in his methylen blue preparations.

Figure 9, A to N, illustrates internal structure of hairs as seen by the writer, using Ehrlich's haematoxylin stain with eosin.

THE SO-CALLED TASTE ORGANS

Several writers, particularly Nagel (*42*), have described certain tiny peglike hairs on the mouth parts of insects as taste organs, but no one has ever demonstrated that they perform such a function. Hochreuther (*15*) found many "Tast- und Geschmackszäpfchen" on the maxillary and labial palpi of *Dytiscus marginalis*. Comstock (*5, p. 132*) briefly summarized the description of such hairs, and said:

> Many experiments have been made to determine the function of the various chemical sense-organs but the results are, as yet, far from conclusive.

The present writer wishes to emphasize the fact that so far as he is aware no recent observer has found gland cells connected with these sense hairs, as claimed by Berlese (*1*).

As shown for adult boll weevils in the preceding pages and for the larvae in the following pages, only two kinds of sense organs—olfactory pores and innervated hairs—were found on the mouth parts. Neither of these seems suitable to act as taste organs, and consequently the writer has come to the same conclusion concerning the boll weevil that he reached several years ago (*32*) in regard to the honeybee. In brief, he does not believe that insects have a true gustatory sense, but since in many cases they must first "taste" certain foods before they can discriminate between them, it seems that their reponses may be similar to ours when we are "tasting" the flavors of particular foods. Long ago physiologists determined that flavors to us are only smells, brought about by placing food in the mouth and then exhaling through the nose.

Since it was impossible to demonstrate by experiment the function of the sense organs on the mouth parts, the writer argued that in view of the fact that bees do not have suitable gustatory organs they can not have a true gustatory sense. Snodgrass (*54, p. 59–63*) well summarizes the writer's results, and adds the following precautionary remarks:

> Most entomologists, probably, will not be convinced by this form of reasoning that insects do not taste, since it places too much reliance on a personal interpretation of the functional possibilities of an observed anatomical structure. It shows, however, that the experimenter who claims a sense of taste for insects must demonstrate it with tests that will clearly avoid a confusion between taste and smell.

THE SO-CALLED AUDITORY ORGANS

Much has been written about the auditory sense of insects, but critics still contend that it has never been demonstrated beyond a doubt that any insect can really hear. Most students on insect behavior believe that insects can hear, yet very few have produced evidence supporting this belief. Rádl (*46*), after summarizing the results of several other investigators on this subject, says that certain

experiments have convinced him that insects have a crude auditory sense. He offers several reasons for this conviction, and concludes by saying that the auditory sense in insects is on a much lower plane of development than that of vertebrates. Its anatomical and physiological antecedents are to be found, not in the tactile organs and contact activities, but anatomically in sense organs which register muscle activities and physiologically in general sensation. That is, he believes that their sense of hearing is a highly refined muscular sense. Turner and Schwarz (*56*) and Turner (*55*) have produced good experimental evidence to show that certain moths really hear, although the present writer is unaware of results equally convincing that beetles can hear; nevertheless, it is common for them to have stridulating devices and chordotonal organs.

The common belief that insects can hear is based on the three following facts: (1) Many have special sound-producing organs; (2) some have so-called auditory organs; and (3) many of the experimental results obtained indicate that insects can hear, although certainly not as we do.

Since the writer could find no reference in literature to the "auditory" organs in a weevil, he has used the two investigations of Lehr (*22*, *23*) as a guide for the work here reported. Lehr, who first studied the anatomy of the sense organs in the elytra and wings of *Dytiscus marginalis*, found a chordotonal organ at the base of the subcostal vein. It lies on the ventral side beneath a group of olfactory pores, is well developed, and has all the parts common to such an organ. In his second work (*23*), Lehr pointed out two other so-called auditory organs in the pedicel or second antennal segment. The Johnston's organ is situated near the distal end of the segment; while the chordotonal organ, lying by its side, arises nearer the proximal end. Both of these organs run lengthwise in the segment and connect with the articular membrane between the second and third segments, but Lehr failed to see all their parts distinctly. Judging from his drawings, the Johnston's organ is the better developed. At this place in the antenna the interior is nearly filled with sense cells.

Johnston in 1855 (*21*) first pointed out a supposed auditory organ in the second antennal segment of the Culex mosquito. This structure, later called after his name, was thoroughly investigated by Child (*3*), who saw it in all the insect orders examined, except the Orthoptera. He found it in Diptera, Hymenoptera, Coleoptera, Neuroptera, Pseudoneuroptera, and Homoptera. He also saw sense organs in the second antennal segments of Orthoptera, but decided that they were not Johnston's organs. These have since been described as olfactory pores by the present writer (*36*), who has also recently described the Johnston's organ in the honeybee (*37*). He did not, however, see a chordotonal organ in the same segment, as mentioned by Lehr and as here reported for the boll weevil.

Externally, there are no signs of an auditory organ in the pedicel or second antennal segment (fig. 4, C) of the adult boll weevil, but sections through the distal end of this segment always show large masses of sense cells. After these masses had been carefully studied under the highest magnification, they were divided into two groups, based on size and structure; but, owing to their minuteness and perhaps to poor fixation and staining, it was not possible to distinguish

all the details ordinarily described for the supposed auditory organs. Since these groups are similar in position and somewhat resemble those described by Lehr in Dytiscus, the writer has called one of them the Johnston's organ (fig. 10, A, J) and the other the chordotonal organ (CO), although perhaps these names should be reversed, or possibly both groups belong to the same organ.

Both groups of sense cells run nearly the full length of the segment. Near the distal end (fig. 10, A) they expand and occupy most of the interior, but near the proximal end (fig. 10, B) they are much smaller and take up less space. One side of each group is always in contact with the hypodermis and seems firmly anchored. The distal ends of

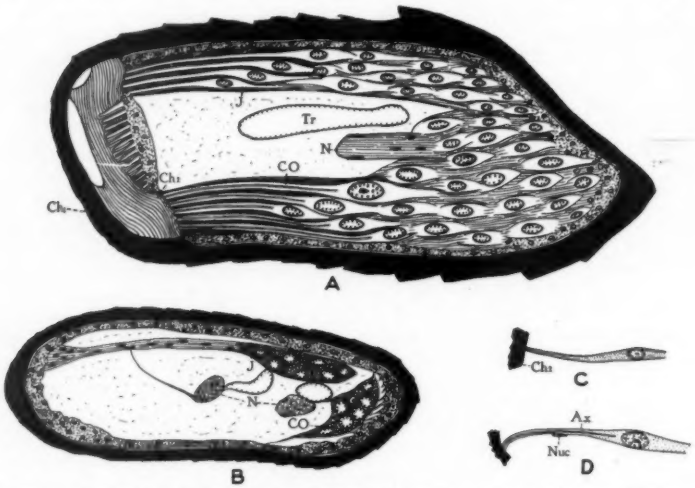

FIG. 10.—The so-called auditory organs of the cotton boll weevil. A and B, two-thirds diagrammatic from oblique longitudinal sections of pedicel (*P* of fig. 4, C), showing Johnston's organ (*J*) and the chordotonal organ (CO). A, from distal end of segment and B, from proximal end. C and D, two of smallest cells from these organs, C being from Johnston's organ and D, from the chordotonal organ. ×564

Abbreviations: Ax, axial tube; Ch_1, primary cuticula; Ch_2, secondary cuticula; N, nerve; Nuc, nucleus; and Tr, trachea

the sense cells are attached to the secondary cuticula (fig. 10, A, Ch_2) of the articular membrane between the second and third segments. A nerve is always near the groups, and branches of it (N) can be traced directly to the sense cells.

The sense cells in the Johnston's organ are usually smaller than those in the chordotonal organ, and seem to be simple in structure (fig. 10, C), while each sense cell in the chordotonal organ apparently bears two additional structures—the axial tube (fig. 10, D, *Ax*) and secondary nucleus (Nuc). This would seem to indicate that the chordotonal organ consists of the various parts common to this type of sense organ.

As already stated, Lehr found a well-developed chordotonal organ in the base of the subcostal vein of Dytiscus. The present writer likewise found a chordlike structure (fig. 6, D, *Co*) in identically the same place in the boll weevil, but no details could be distinguished.

Since it appears nearly structureless in the few sections showing its presence, we may infer that it is either a rudimentary organ or had not had time to develop fully when the weevils emerged.

Graber (*10*), after finding chordotonal organs in many of the insect orders, was convinced that the integument of insects, like the tympanum of vetebrates, is well adapted to be set in vibration by sounds, and that the terminal nerve connections of these organs are the sound receptors.

During the past 75 years, the stridulating or sound-producing organs of insects have been much discussed, but still we do not know whether the noises made by them are intentional or unintentional. Darwin (*7*) calls these organs sexual characters, but he has much difficulty in finding examples to prove that they are so. If all our present information were carefully analyzed, however, with this object in view, we might be able to produce many good examples to support his belief.

In regard to beetles Darwin (*7, p. 310, 311, 312*) says:

> Nevertheless, the power of stridulating is certainly a sexual character in some few Coleoptera. * * *. In the case of the Heliopathes and Oryctes there can hardly be a doubt that the males stridulate in order to call or to excite the females; but with most beetles the stridulation apparently serves both sexes as a mutual call. Beetles stridulate under various emotions, in the same manner as birds use their voices for many purposes besides singing to their mates. The great Chiasognathus stridulates in anger or defiance; many species do the same from distress or fear, if held so that they can not escape; by striking the hollow stems of trees in the Canary Islands, Messrs. Wollaston and Crotch were able to discover the presence of beetles belonging to the genus Acalles by their stridulation. Lastly, the male Ateuchus stridulates to encourage the female in her work, and from distress when she is removed. Some naturalists believe that beetles make this noise to frighten away their enemies; but I can not think that a quadruped or bird, able to devour a large beetle, would be frightened by so slight a sound * * *. Finally, it is probable that the two sexes of many kinds of beetles were at first enabled to find each other by the slight shuffling noise produced by the rubbing together of the adjoining hard parts of their bodies; and that as those males or females which made the greatest noise succeeded best in finding partners, rugosities on various parts of their bodies were gradually developed by means of sexual selection into true stridulating organs.

So far as known to the writer, no one has reported stridulating organs in boll weevils, nor has anyone heard noises made by these insects, yet stridulating organs may be present and the adults may make noises which have never attracted the attention of any observer. Darwin cites cases of other curculionids having stridulating organs with which they are able to make audible noises, and Westring (*58*) as early as 1847 reports finding these organs in certain curculionids. In these weevils the rasps are located on the inferior surface of the elytra, near the apices, or along their outer margins, and the edges of the abdominal segments serve as the scrapers. This arrangement is the reverse of what usually occurs in other beetles. These weevils make a loud noise by rapidly rubbing the tips of the abdomen on the ends of the elytra. As already quoted by Darwin, Wollaston (*59*) called *Acalles argillosus* in the Canary Islands a musical curculionid, and another observer reports five other species of Acalles, one of which is very minute, as being musical in the Madeira Islands. All of these species have stridulating organs.

EYES AND LIGHT EXPERIMENTS

The two compound eyes (fig. 4, A, *E*) of the adult boll weevil are present; but, as reported by other writers, the ocelli are apparently absent. The faceted eye is small, though moderately convex. The number of facets in the eyes of one male and one female were counted; they ranged from 400 to 448, with an average of 433 per eye; and there was practically no sexual difference. According to Müller's mosaic theory of sight in insects, which most entomologists have accepted, the larger and more convex the eyes the larger will be the visual field, and the smaller and more abundant the facets the sharper and more distinct will be the image. Using the preceding information as a basis for speculation, we may infer that the visual field of the boll weevil is small and its image of objects is neither sharp nor distinct. From this statement we may also infer that the boll weevil probably can not, merely by sight, distinguish from a distance cotton plants from certain other plants, and furthermore that it may not be able by sight alone to distinguish the various parts of a cotton plant.

Many sections of the eyes were made, but after a preliminary study of them no unusual structures were noted that would modify the generally accepted view of insect vision. If one may judge from the large amount of pigment present, the boll weevil has day eyes and not night eyes. The subject of day and night eyes is well summarized by Comstock (*5, p. 142*).

A median ocellus (fig. 4, A, Oc) was finally found in the boll weevil after a diligent search under a high magnification. It is very small, extremely oblong, and lies nearly buried in a slit between the compound eyes; but viewed from the inner side of caustic-potash preparations it appears much larger, and is nearly round.

The only light experiments conducted by the writer were a few preliminary ones to verify the statement that the boll weevil is positively phototropic to daylight. No artificial lights were tested; although it might be possible to attract weevils to lights closely imitating daylight. Ever since the advent of this insect to cultivated cotton fields, and even up to the present time, there has been a belief that it might be attracted to artificial lights and then killed. Hunter and Hinds (*17, p. 160*) and Hunter and Pierce (*18, p. 154*) assure us that there is no scientific foundation for this belief. Thousands of insects, including many weevils similar in appearance to the boll weevil, have been attracted to lights in cotton fields, but as yet no scientist has found a single cotton boll weevil among them.

Hunter and Pierce (*18, p. 43*) report the results of some preliminary experiments conducted in 1907 to ascertain the color sense of boll weevils. Of the 14 shades and colors used, the 3 most attractive were light blue, dark green, and light pink, the light blue being most preferred.

Lutz (*25, p. 265*), discussing the color sense of insects, says:

> Hess may be right in believing that insects are totally color blind. Probably Frisch is more nearly correct in saying that they can distinguish all of the colors except red and certain greens as colors, those two of our colors appearing to them as darker or lighter grays; in other words, that their color vision is similar to that of some partially color-blind humans.

Lutz determined that the flowers and occasionally other parts of certain plants emit ultra-violet rays, and that flower-visiting insects respond to ultra-violet light, but he did not use the cotton plant

and the boll weevil; although it is possible that the cotton plant emits these rays and perhaps the boll weevil responds to them. Lutz (*25, p. 271, 278, 280*), further states:

> It has been shown that, in addition to the colors which man can see, some flowers are ultra-violet, while others are not at all ultra-violet, and still others have an ultra-violet pattern. It has also been shown that flower-visiting insects can see ultra-violet as well as or even better than they can see the rays perceived as light by man. The effect of this work is to add one more color—and apparently an important one—to the list of those which plants use in facilitating the visits of insects, if plants do use colors for such a purpose.
>
> All of the colors of the spectrum from red to ultra-violet, both included, are to be found in light reflected by one flower or another. Of these waves of light reflected by flowers, those of relatively great length, red to green, are more common than those of shorter length, blue to ultra-violet. Flower-visiting insects do not see red to green as well as they do blue to ultra-violet.
>
> It is also shown that a wide range of flower-visiting insects are photopositive to ultra-violet. It appears as though they see ultra-violet better than they do colors that seem bright to us. Accordingly, ultra-violet becomes quite as important in discussions of the relation between floral colors and insects as any other color.

OTHER SENSES

Among the general sensations of insects might be mentioned those of temperature, humidity, direction, hunger, fear, pain, and statical conditions. Very little is known about these senses in insects in general, and practically nothing about them in the boll weevil.

The sense of temperature in the boll weevil, judging from its reactions to different degrees of heat and cold, is probably well developed, but, as in other insects and even in ourselves, there are probably no special sense organs to receive thermal stimuli. The subhypodermal nerve plexus, if it is present in the adult weevil, could easily perform this function. Humidity, which is closely related to temperature, also has much to do with the behavior of this insect.

In this connection might also be mentioned some of the ingenious but absurd mechanical devices which have been invented from time to time for the purpose of eradicating the boll weevil. Among these the most common are "light" traps, one even being pulled through the fields on wheels; another is planned to attract the weevils by sounds; and still another would kill the weevils with a beam of high pitched inaudible sound waves of great intensity.

One of the newest ideas about the means of communication among insects is wireless telegraphy. This suggestion seems to have been first made by Fabre (*8, p. 194–199*) to explain how male moths find their mates from long distances, but his experiments convinced him that moths do not communicate by this means; next he predicted that science would provide us with a radiography of odors, after the pattern of the Röntgen rays, although Forel (*9*) says that Fabre's own results contradict this view. The next idea along this line was suggested by Riley (*50, p. 38–41*), who used the word telepathy to explain how insects communicate from long distances, but still he had no faith in this view.

Now, since we are able to communicate among ourselves so readily by radio, this means has been suggested as possible for insects. The writer can find no authentic data on this subject, but in some newspaper reports which recently appeared it is stated that one authority claims that man and most other animals constantly emit

N rays or electric rays which are used in communication. He says in part:

> Look at the birds which fly at night, insects, blind fish at the bottom of the sea—all are irresistibly drawn toward their prey not so much by sight or odor, but by the action of the radiations which direct them and to which they submit automatically.

In regard to insects he imagines that these tiny "radio waves" are received by the antennae. Another report, this one given out by Science Service, of Washington, D. C., says in part:

> Animals and plants while living give off rays similar to those emitted by the metal radium, according to reports made to the French Academy of Sciences by Albert Nodon.

Mast (*39*) experimented with fireflies and concluded that smell played no part in bringing the sexes together, but that the sexes communicate and are attracted to each other by means of their flashes of light. Singh and Maulik (*52*) determined that the so-called "phosphorescence" of the fireflies would affect the photographic plate through wood, dark brown leather, black paper, or flesh, and conclude:

> The light emitted by the insect cannot therefore be taken as phosphorescent. It may be, perhaps, premature to conclude that some of the rays emitted by the insects are X-rays, but it may be safely asserted that these rays are, at least, similar to X-rays and ultra-violet light in so far as they render certain opaque media transparent and are intercepted by glass.

Harvey (*11, p. 61, 62*) says:

> Although Muraoka (1896) and Singh and Maulik (1911) have described radiations coming from fireflies which would pass opaque objects and affect a photographic plate, * * * the existence of such radiation has been denied by Suchsland (1898), Schurig (1901), and Molisch (1904).
>
> There is, then, no specific emission of X rays or similar penetrating radiation from luminous tissues which will affect the photographic plate through opaque screens.

A letter from E. N. Harvey to the writer, dated June 7, 1925, contains his latest ideas on this subject, including the possibility that boll weevils might give off rays which can be perceived by themselves but not by us. He states:

> I do not think there is any evidence that animals give off X rays or any other kind of peculiar radiation. Short ultra-violet radiation is very destructive to living matter, so there is not much chance of wave length shorter than 3,000 Å being emitted, but I see no reason why ultra-violet between 4,000 Å and 3,000 Å should not be produced. We do not know definitely of a case, so that the matter is merely speculation.

RECOGNITION AMONG BOLL WEEVILS

Hunter and Hinds (*17, p. 76*) found that the male boll weevil was unable to recognize the female at a much greater distance than an inch, and that the attraction is apparently effected by smell. Field observations, as well as laboratory ones, seemed to show that the sexes are attracted only when they meet, as they are likely to do either on the stems or squares of the plant.

While watching female weevils both in the field and in confinement, the writer observed that they seemed to pay little attention to one another; but such is not usually the case where the opposite sex is concerned. The males in confinement usually do not lose an opportunity to mate, and should no female be handy, a male may often be

seen trying to copulate with another male. This behavior of either sex does not necessarily imply that the females do not know one another, or that the males can not distinguish the sexes, because we can cite many similar cases among other animals; for example, cows in heat or rut often try to copulate with one another.

If boll weevils do not communicate with one another by telephathy, radio, or by any other type of radiation, as mentioned above, then there are left perhaps only three other means—sight, touch, and smell. At short distances the weevils may be able to distinguish many other insects and perhaps inanimate objects by sight alone, and when in contact may partially recognize one another by sight and touch; but if they are similar to other insects and the higher animals, smell probably plays a more important rôle in recognition than do the other senses.

If boll weevils recognize one another by means of smell, then they must produce odors which can be smelled. Insects, as well as the higher animals, have glands connected with their sexual organs, and those in the boll weevil might serve for sexual recognition.

The unicellular glands found widely distributed over the entire body surface are, in point of abundance, as follows: On the elytra (fig. 16, E, *po*) and antennae (fig. 4, C; fig. 8, PorGl), very numerous; on the beak or snout (fig. 7, A), numerous; on the wings (fig. 16, D, *po*) and ventral side of the abdomen, fairly numerous; on the head capsule, mouth parts (fig. 3, A, C, D, and F), thorax, legs (fig. 16, C), and dorsal side of the abdomen, few.

The external appearance of these glands is shown in Figure 5, A and B, and the internal anatomy in Figure 5, M, N, O, and Figure 16, B and F. It will be noted that the gland cell (GlC) is much larger than the hypodermal cells (HypC) and that an ampulla (Am) is present. The secretion collects in the ampulla, passes through the cell wall into the pore canal (PorC), then into the reservoir (Re), and finally to the outside through the efferent tube (Ef). It was not possible to decide definitely whether this tube is open or closed at its inner end; theoretically, it should be closed, but in many cases it seemed to be open. The drawings show it both ways. It is shaped like a finger or a test tube, and its walls are so thin that it really does not matter whether it is closed or open, for in all probability the secretion can pass through its walls by osmosis.

Several years ago the writer (*33*) collected the literature pertaining to the scent glands or scent-producing organs in insects and classified them on the basis of their distribution. The simplest and commonest type found among beetles and a few other insects is composed of the unicellular glands widely distributed over the body surface. This type is well illustrated on pages 38 to 40 of the above paper, although it is not up to date.

Parts of the body surface of live weevils appear shiny, but not wet, as is often observed in certain other beetles. Several years ago the writer (*31*) made a careful study of this question in the squash beetle (*Epilachna borealis*). These beetles always appear wet, and the more they are irritated the wetter they become. The secretion, which comes from numerous hypodermal glands widely distributed over the integument, is easily seen on the body surface. It has a bitter taste and emits an odor repugnant to us.

N rays or electric rays which are used in communication. He says in part:

> Look at the birds which fly at night, insects, blind fish at the bottom of the sea—all are irresistibly drawn toward their prey not so much by sight or odor, but by the action of the radiations which direct them and to which they submit automatically.

In regard to insects he imagines that these tiny "radio waves" are received by the antennae. Another report, this one given out by Science Service, of Washington, D. C., says in part:

> Animals and plants while living give off rays similar to those emitted by the metal radium, according to reports made to the French Academy of Sciences by Albert Nodon.

Mast (*39*) experimented with fireflies and concluded that smell played no part in bringing the sexes together, but that the sexes communicate and are attracted to each other by means of their flashes of light. Singh and Maulik (*52*) determined that the so-called "phosphorescence" of the fireflies would affect the photographic plate through wood, dark brown leather, black paper, or flesh, and conclude:

> The light emitted by the insect cannot therefore be taken as phosphorescent. It may be, perhaps, premature to conclude that some of the rays emitted by the insects are X-rays, but it may be safely asserted that these rays are, at least, similar to X-rays and ultra-violet light in so far as they render certain opaque media transparent and are intercepted by glass.

Harvey (*11, p. 61, 62*) says:

> Although Muraoka (1896) and Singh and Maulik (1911) have described radiations coming from fireflies which would pass opaque objects and affect a photographic plate, * * * the existence of such radiation has been denied by Suchsland (1898), Schurig (1901), and Molisch (1904).
>
> There is, then, no specific emission of X rays or similar penetrating radiation from luminous tissues which will affect the photographic plate through opaque screens.

A letter from E. N. Harvey to the writer, dated June 7, 1925, contains his latest ideas on this subject, including the possibility that boll weevils might give off rays which can be perceived by themselves but not by us. He states:

> I do not think there is any evidence that animals give off X rays or any other kind of peculiar radiation. Short ultra-violet radiation is very destructive to living matter, so there is not much chance of wave length shorter than 3,000 Å being emitted, but I see no reason why ultra-violet between 4,000 Å and 3,000 Å should not be produced. We do not know definitely of a case, so that the matter is merely speculation.

RECOGNITION AMONG BOLL WEEVILS

Hunter and Hinds (*17, p. 76*) found that the male boll weevil was unable to recognize the female at a much greater distance than an inch, and that the attraction is apparently effected by smell. Field observations, as well as laboratory ones, seemed to show that the sexes are attracted only when they meet, as they are likely to do either on the stems or squares of the plant.

While watching female weevils both in the field and in confinement, the writer observed that they seemed to pay little attention to one another; but such is not usually the case where the opposite sex is concerned. The males in confinement usually do not lose an opportunity to mate, and should no female be handy, a male may often be

seen trying to copulate with another male. This behavior of either sex does not necessarily imply that the females do not know one another, or that the males can not distinguish the sexes, because we can cite many similar cases among other animals; for example, cows in heat or rut often try to copulate with one another.

If boll weevils do not communicate with one another by telephathy, radio, or by any other type of radiation, as mentioned above, then there are left perhaps only three other means—sight, touch, and smell. At short distances the weevils may be able to distinguish many other insects and perhaps inanimate objects by sight alone, and when in contact may partially recognize one another by sight and touch; but if they are similar to other insects and the higher animals, smell probably plays a more important rôle in recognition than do the other senses.

If boll weevils recognize one another by means of smell, then they must produce odors which can be smelled. Insects, as well as the higher animals, have glands connected with their sexual organs, and those in the boll weevil might serve for sexual recognition.

The unicellular glands found widely distributed over the entire body surface are, in point of abundance, as follows: On the elytra (fig. 16, E, *po*) and antennae (fig. 4, C; fig. 8, PorGl), very numerous; on the beak or snout (fig. 7, A), numerous; on the wings (fig. 16, D, *po*) and ventral side of the abdomen, fairly numerous; on the head capsule, mouth parts (fig. 3, A, C, D, and F), thorax, legs (fig. 16, C), and dorsal side of the abdomen, few.

The external appearance of these glands is shown in Figure 5, A and B, and the internal anatomy in Figure 5, M, N, O, and Figure 16, B and F. It will be noted that the gland cell (GlC) is much larger than the hypodermal cells (HypC) and that an ampulla (Am) is present. The secretion collects in the ampulla, passes through the cell wall into the pore canal (PorC), then into the reservoir (Re), and finally to the outside through the efferent tube (Ef). It was not possible to decide definitely whether this tube is open or closed at its inner end; theoretically, it should be closed, but in many cases it seemed to be open. The drawings show it both ways. It is shaped like a finger or a test tube, and its walls are so thin that it really does not matter whether it is closed or open, for in all probability the secretion can pass through its walls by osmosis.

Several years ago the writer (*33*) collected the literature pertaining to the scent glands or scent-producing organs in insects and classified them on the basis of their distribution. The simplest and commonest type found among beetles and a few other insects is composed of the unicellular glands widely distributed over the body surface. This type is well illustrated on pages 38 to 40 of the above paper, although it is not up to date.

Parts of the body surface of live weevils appear shiny, but not wet, as is often observed in certain other beetles. Several years ago the writer (*31*) made a careful study of this question in the squash beetle (*Epilachna borealis*). These beetles always appear wet, and the more they are irritated the wetter they become. The secretion, which comes from numerous hypodermal glands widely distributed over the integument, is easily seen on the body surface. It has a bitter taste and emits an odor repugnant to us.

What is the function of this secretion? When it is repellent or distasteful to the insect's enemies, we say that its primary function is for protection and its secondary function probably for recognition. Since the boll weevil does not have a wet body surface, its hypodermal glands certainly do not secrete as copiously as do those in certain other beetles, and in all probability this secretion is not for protection; in which case its primary function must be for recognition. A single boll weevil emits little or no odor noticeable to a person, but when several are confined in a small box or bottle, they emit a characteristic and rather pronounced odor.

Scent glands of this type do not have reservoirs on the body surface to hold the secretion and so prevent it from evaporating too rapidly, but such structures are common in other types. Wherever these glands occur the secretion runs to the exterior and spreads in a film over the chitin and then constantly and perhaps quickly evaporates, giving off its own characteristic odor. However, on the elytra of boll weevils there are peculiar structures called punctures, which might serve as reservoirs for this secretion, provided it spreads sufficiently to reach them. On all parts of the surface (fig. 16, C to F, *po*), except on the wings, where these gland pores occur, they are associated with the hairs, one usually lying at or near the base of each hair (C and E). On the dorsal surface of an elytron there are numerous club-shaped or protective hairs (*j*); numerous gland pores (*po*); and several longitudinal rows or striae of slit-shaped pits, the punctures (*n*). Each puncture consists of an oblong, heavily pigmented border (*l*), a lengthwise slit (E and F, *n*), and a tiny hair (*m*), which lies flatly over and nearly closes the opening of the slit or puncture. The punctures on the right elytron of a male were counted. They lie in 10 rows, ranging from 25 to 39 per row, making a total of 319 for the elytron. What can be the function of these punctures? The writer does not know, unless they serve as reservoirs for some secretion. The minute hair is always present in the same place, and could well serve as a door for these pits.

SENSE ORGANS OF LARVAE OF BOLL WEEVILS

The larvae of boll weevils are white, delicate, and legless grubs, being well protected by the covering of the bolls or squares. Since they spend all their lives inside a dark house, well surrounded by food, the question may properly be asked, "Do they need sense organs?" It is possible that they do not eat all parts of the food surrounding them, in which case they must distinguish the edible from the nonedible by touch, taste, or smell; on the other hand, if they do eat all of the food surrounding them and have sense organs suitable for this purpose, then we conclude that these organs are useless and have only a phylogenetic significance.

OLFACTORY PORES

The olfactory pores on five full-grown larvae were carefully studied. They were found practically constant in position and number, and since those on larva No. 4 were most critically examined and then drawn, they will be described in detail.

No groups of pores were found, but the isolated ones (fig. 11, A to F, *a* to *z*) were usually arranged in pairs; one pore being on the right

side and its mate on the left side of a median line. Ten pairs (*a* to *j*) lie on the head capsule, pore *g* of these being on the microscopic antennae; 3 or 4 pores (*k*) on each mandible; 5 pairs (*l* to *o*) on the labium; 1 pair (*p*) on the mentum; 1 pair (*q*) on the submentum; 1 pair (*r*) on the labrum; 1 pair (*s*) on the clypeus; 8 pores (*t* to *y*) on each maxilla; 2 pairs (*z*) on the second thoracic segment; and 4 pairs (fig. 12, B) on what appears to be the epipharynx.

The external structure (fig. 12, A and B) and internal structure (fig. 12, D to H) of the pores on the larvae are like those already

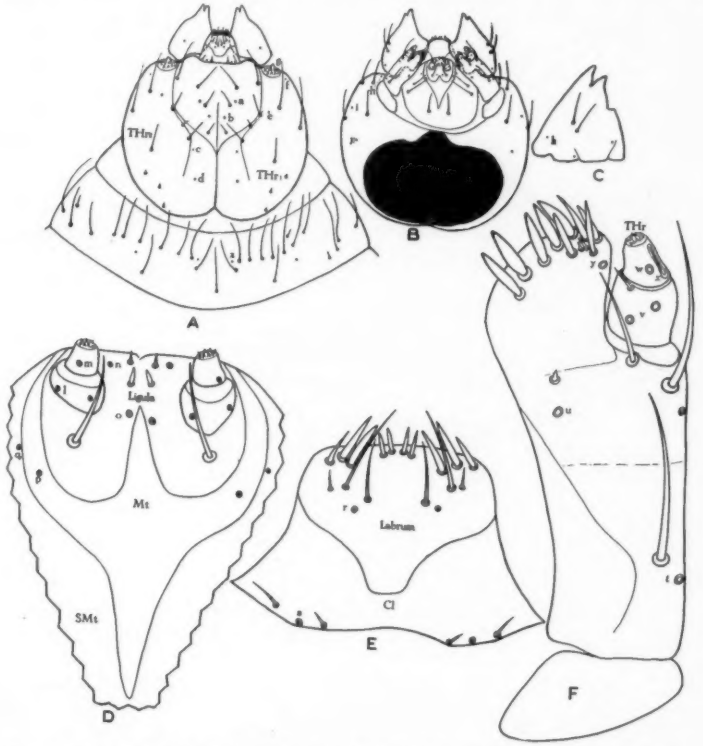

FIG. 11.—Disposition of olfactory pores (*a* to *z*) and hairs on larva of boll weevil. A, dorsal view of head and first two thoracic segments; B, ventral view of head; C, outer surface of right mandible; D, ventral view of labium, mentum (Mt), and portion of submentum (SMt); E, dorsal view of labrum and clypeus (Cl); and F, ventral view of maxilla. A and B, × 31; C, × 52; D to F, × 190

described for the adult weevil. On the maxillary palpus (fig. 3, B) of the adult there are five slit-shaped pores, but on that of the larva there is only one (fig. 11, F, and fig. 12, A, *x*).

OLFACTORY PORES OF OTHER LARVAE

No one, except the writer, has apparently identified the olfactory pores as the Hicks' organs in any larva, although it is evident that they have been seen by various systematists, for Schiødte (*51, 1872–73*)

figured them on the antennae and labial palpi of staphylinid larvae, on the legs of scarabaeid larvae (*51, 1874*), on the maxillae of lucanid larvae, and on other coleopterous larvae. Böving (*2*) figured a few of them near the spiracles of a coccinellid larva, and Cotton (*6*) showed a very few on the mouth parts of different curculionid larvae. Nagel (*42*) saw two of these pores on the maxillary palpus of a larva of a stonefly (*Perla bicaudata* Panzer); very few on the antennae and labrum of a lepidopterous larva (*Antheraea pernyi* Guér.), and a few widely distributed on the antennae, maxillae, and labium of a coleopterous larva (*Dytiscus marginalis*). Nagel called them "Gruben ohne Kegel" and made sections through them, but did not suggest a function for them, because he failed to understand their internal anatomy. The present writer (*35*) made a careful study of these pores in 30 species of lepidopterous larvae, and determined experimentally that these larvae respond to chemical stimuli, although no experiments were performed to determine the function of the pores.

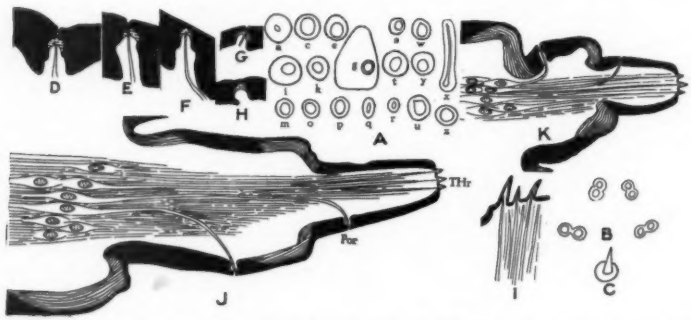

FIG. 12.—A to C, external structure of olfactory pores and tactile hairs of larva of boll weevil. In A the olfactory pores are lettered *a* to *z* (with pores *b*, *d*, *f*, *h*, *j*, *l*, *n*, and *r* omitted), their position being indicated in Figure 11, A to F; *c*, *e*, and *i*, from head capsule; *g*, antenna with its pore; *k*, from mandible; *m*, from labial palpus; *o*, from ligula; *p*, from mentum; *q*, from submentum; *r*, from labrum; *s*, from clypeus; *t*, from stipes; *u*, from palpifer; *w* and *x*, from palpus; *y*, from lacinia of maxilla; and *z*, from second thoracic segment. B, olfactory pores (too near one another) from epipharynx? C, one of smallest tactile hairs (THr_1, fig. 11, A) on head. Internal anatomy of olfactory pores and innervated hairs of larva of boll weevil. D to H, olfactory pores; D, from labrum; E, from mandible; F, from antenna; G, from maxillary palpus; and H, from labial palpus. I, innervated sense pegs around base of antenna. J and K, from longitudinal sections of maxillary palpus and labial palpus, respectively (mostly diagrammatic), showing innervation of olfactory pores (Por) and sense pegs (Thr). × 492

The writer (*34*) carefully studied the morphology of the olfactory pores in the larvae of *Cotinis nitida* L. Two types of pores were found, single and compound. The compound were observed only on the terminal segments of the long antennae. Recently the same type has been seen similarily located in the larvae of a May beetle (*Lachnosterna* sp.) and the Japanese beetle (*Popillia japonica* Newm.), two other scarabaeids. The single organs (isolated pores) in Cotinis were found on the antennae, mouth parts, head, thorax, and legs. The average total number of pores counted in both types was 1,359. Many drawings of the two types were prepared and published, but photomicrographs, not previously published, are here presented (figs. 13–15) to convince the reader that the sense fibers of these organs actually enter minute pores, called pore apertures. Of course the writer is well aware that such a view is contrary to the accepted belief concerning the presence of pores in the integument of insects; nevertheless he is thoroughly convinced that the ends of these nerves

come in contact with the external air. Just how they end in these pores can not be stated, because they are so small and indistinct that no definite details can be discerned. In the best sections there does not appear to be any membrane, however thin, between the end of the sense fiber and the outside air, although it must be granted that the tip of this fiber is covered by its own cell wall. A careful study of these photomicrographs will show how the two types of pores appear under a high magnification, but particular attention is directed to Figure 13. C shows the complete innervation of an olfactory pore. B is a section of an olfactory pore obtained by using an ordinary photographic plate. Attention is called to the sense fiber (*c*) which passes through the chitinous cone, and stops at A, *a*. A is a section of the same olfactory pore, obtained by using a photographic plate sensitive to yellow. Attention is here called to the pore aperture (*p*) or pit passing from the exterior to the point *a*, where the sense fiber (*c*) enters the pit.

OLFACTORY ORGANS IN MAN AND THE HIGHER ANIMALS

Reference to recent textbooks such, for example, as Howell's "Physiology" (*16*) and Piersol's "Human Anatomy" (*43*) will quickly show the reader that the protoplasmic cilia of the olfactory cells in man and the higher animals also come in contact with the outside air. Except in this type of olfactory organ, the microscopic sense cilia are constantly covered with mucus, and in order to produce a sensation of smell the odoriferous particles are first dissolved in this mucus. It is generally believed by the layman that these cilia and their cells are covered by a thin and moist membrane through which the odoriferous air must pass; but the authorities on this subject tell us that wherever the olfactory cells occur in the nasal passages, the epithelium consists of two chief constituents—supporting cells and olfactory cells. The olfactory cells project slightly beyond the general level of the epithelium and each bears six to eight minute stiff cilia, often called olfactory hairs. Mucous glands, lying just beneath the outer or olfactory layer, are very numerous; their orifices are barely distinguishable by the unaided eye; and they keep the outer surface of the membrane well covered with mucus all the time.

The end of each sense fiber in the olfactory pores of insects might be compared to a cilium on an olfactory cell of man. Since the sense cells in insects are modified hypodermal cells, they may still retain a small part of their original secretory use, in which case they would be able to keep the tip ends of their sense fibers sufficiently moist so that the odoriferous particles touching them would be dissolved. If this condition exists, then the olfactory apparatus in insects and the higher animals is very similar in structure, and certainly must be so in function.

TACTILE HAIRS

Since the hairs on the larvae of the boll weevil are comparatively scarce and widely scattered, they were not easily found in sections; consequently many of those called sense hairs and sense bristles in Table 7 have been thus classified merely from their external structure; although the innervation of those at the tips of the maxillary and labial palpi (fig. 11, D, F, and fig. 12, J, THr, and K), and at the base of the antennae (fig. 12, I) has been thoroughly studied.

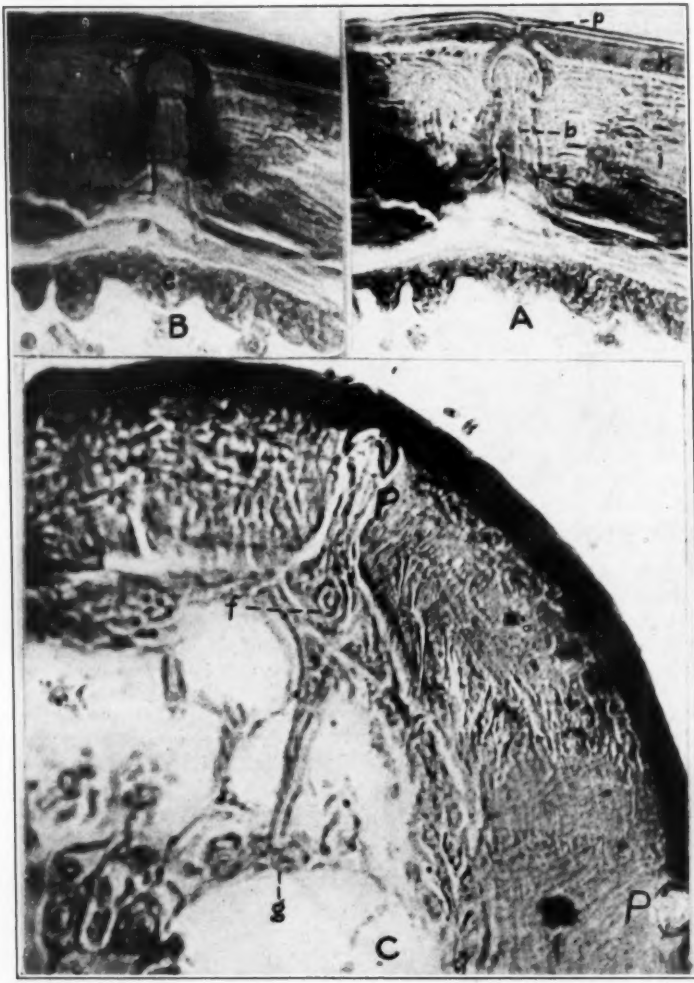

FIG. 13.—Photomicrographs showing olfactory pores in antenna of larva of *Cotinis nitida*. A, section of olfactory pore obtained by using a photographic plate sensitive to yellow. Attention is called to the pore aperture (*p*) or pit passing from the exterior to the point *a*, which is the end of the sense fiber. It is clear that no membrane separates the end of this fiber from the outside air. B, section of same olfactory pore obtained by using an ordinary photographic plate. Attention is called to the sense fiber (*c*) which passes through the chitinous cone and stops at A, *a*. C, portion of cross section, showing complete innervation of another olfactory pore (P); *f*, sense cell, and *g*, branches of nerve. *d*, wall of olfactory pore; *e*, hypodermis; *h*, primary cuticula; and *i*, secondary cuticula. × 1,450

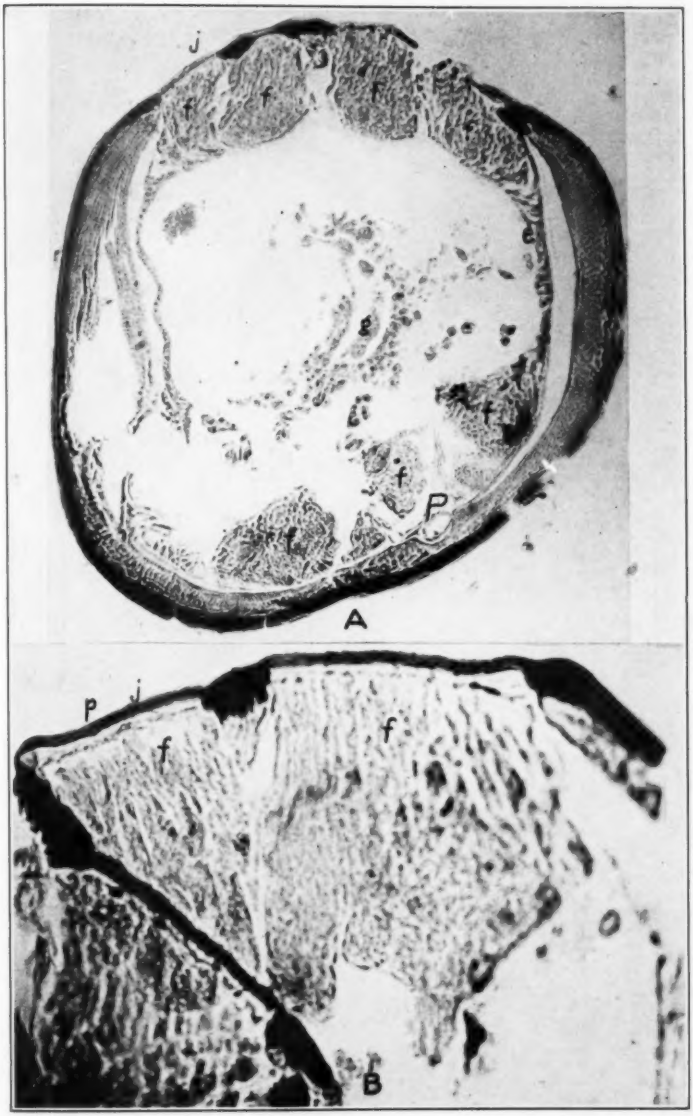

FIG. 14.—Photomicrographs from sections through tips of antennae of *Cotinis nitida*, showing internal anatomy of compound olfactory organs, including *e*, hypodermis; *f*, groups of sense cells; *g*, branches of nerve; *j*, plate over sense cells; *p*, pore through plate; and P, a single olfactory pore. A, cross section, and B, portion of longitudinal section

FIG. 15.—Photomicrographs showing disposition of olfactory organs on legs and antennae of *Cotinis nitida*. A, four single olfactory pores (two not in focus) and a hair (at upper left corner) on outer surface of trochanter; B, ventral view of distal end of antenna, showing three compound olfactory organs, and just beneath them four olfactory pores (only one shows plainly); C, a greater magnification of the above three compound olfactory organs. Attention is called to the pore apertures (small circles) in their plates

Hilton (*14*), who used the section method for tracing nerves to hairs and also the method of injecting methylene blue just beneath the integument of live larvae, mostly Lepidoptera, summarizes his results as follows:

> Lepidopterous larvae are clothed with hollow hairs, each of which is supplied by a bipolar nerve cell, a process of which penetrates a short distance into the hair and probably terminates before reaching the tip. In most species all body hairs are sensory; large hairs are supplied by large bipolar nerve cells, and small ones by smaller bipolar cells. Under the hypodermis of caterpillars there is a system of multipolar cells more or less intimately connected with nerve cells and fibers which stain lighter than the larger nerves and are closer to the hypodermis than the other cells and fibers. Nerves from bipolar sensory nerve cells go to the central nervous system, run to the ganglia, leaving at once to follow on the outside of the connectives cephalad, forming a well-marked sensory tract. Motor nerves—those that go to muscles—seem to come directly from the central cell areas of the ganglia. Almost the only sensory termination of nerves on the body of insects is by means of hairs.

Hilton furthermore states:

> In the larvae of May beetles the hairs of the body surface are supplied with bipolar nerve cells.

In the mind of the present writer it is very questionable whether *all* the hairs on any larva are actually sensory.

Zawarzin (*60*) used a similar method of injecting methylene blue into live larvae of three species of Aeschna, and he also used the section method for studying the chitinous apparatus of the sense organs. Very often he had no success with the methylene-blue method, but after much experience he was able to trace nerves to the body hairs.

The present writer, using live larvae of the boll weevil, tried the injection method used by Zawarzin, but had no success in the few preliminary tests conducted, and did not make further attempts because of the lack of live larvae at the time.

TABLE 7.—*Disposition of sense organs on larva of cotton boll weevil*

Divisions	Sense hairs	Sense bristles	Sense pegs	Olfactory pores	Eyes
Head capsule	Several	Several ?	None	18	Vestigial ocelli.
Antenna	None	None	7	1 at base	
Mandible	do	2 ?	None	3 or 4	
Labium	2	2	Several	3 on palpus, 4 on ligula.	
Mentum	None	None	None	2	
Submentum	do	do	do	2	
Labrum	2	4	10 ?	2	
Clypeus	2	2	None	2	
Maxilla	2	Several ?	Several	1 on stipes, 2 on palpifer, 1 on lacinia, 4 on palpus.	
Thorax	Few on second segment, probably few on other segments.	Several on second segment, probably several on other segments.	None	4 on second segment.	
Epipharynx ?	None	None	Few	8	

OTHER SENSE ORGANS

Besides the olfactory pores and tactile hairs (fig. 11) already described, we should hardly expect to find other sense organs in the larva of the cotton boll weevil; but vestigial ocelli, nevertheless, were found in sections through the head near the antennae. Sphere-

shaped masses of dark-brown granules, the rudiments of the internal anatomy of these eyes, were present, but no convex cornea was visible; however, the chitin above these granular masses was thin and unpigmented, and thus this rudimentary eye might be able to distinguish light from darkness.

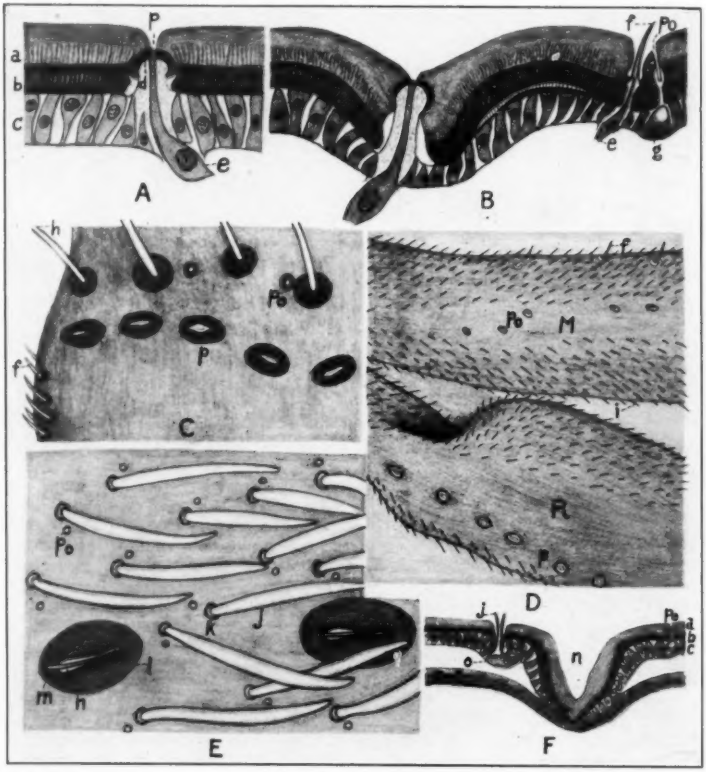

FIG. 16.—Internal and external structure of body wall of boll weevil: All figures are mostly diagrammatic. A, section from tibia; B, section from trochanter; C, superficial view from trochanter of front leg, showing Group 7 of olfactory pores; D, superficial view from wing, showing portions of media (*M*), radius (*R*), and six pores of Group 6; E, superficial view from elytron; and F, section passing crosswise through elytral puncture (*n*)

a, Primary, and *b*, secondary cuticula; *c*, hypodermis; *d*, chitinous cone; *e*, sense cell; *f*, tactile hair (No. 1); *g*, gland cell; *h*, noninnervated hair on leg; *i*, prickle or noninnervated psuedohair on wing; *j*, large club-shaped, noninnervated hair on elytron; *k*, hair socket or alveolus; *l*, border of puncture; *m*, noninnervated hair lying over opening (*n*) of elytral puncture; *o*, trichogen cell forming club-shaped hair (*j*); *p*, olfactory pore; and *po*, pore of gland. A and B, × 714; C to F, × 271

No attempt was made to find chordotonal organs in the larva. They may be present, for Comstock (*5, p. 148*) says:

> Chordotonal organs have been observed in so many larvae that we may infer that they are commonly present in larvae. These organs are very simple compared with those of certain adult insects.

Credit is due Schiødte (*51, 1869*) for describing and figuring chordotonal organs for the first time. He found them on the thoracic segments of buprestid larvae. Graber (*10*) described chordotonal

organs in the larvae of Dytiscus, and Hess (*12*) has given us a good description of them in cerambycid larvae. In 1874 Schiødte (*51, p. 88*) gave an account of the stridulating organs in the larvae of certain Scarabaeidae and Lucanidae.

Larvae, like adult insects, certainly have general senses, but nothing definite is known about them. The subhypodermal nerve plexus, described by Hilton (*14*) and several earlier writers, may serve as receptors for temperature and humidity.

HOW PLANTS ATTRACT INSECTS BY SMELL

It was assumed in the introduction that cotton plants emit odors which attract boll weevils, but so far no proof in support of this assumption has been given. Although the writer has not been in a position to work extensively with boll weevils and cotton plants, he has nevertheless carried on the same line of work using other insects and their host plants. Only a very brief summary of the results of these experiments can be given here.

As flowers are generally more or less fragrant, so the foliage of most plants also emits odors. These odors are usually weak, judged by the human olfactory sense, but to the insect which feeds upon the foliage such may not be the case. Of course, it was impossible to test the response of the insect to the odor of its host plant in the field, because the interfering factors could not be controlled. Consequently it was necessary to develop a special apparatus to be used in the laboratory. A full description of this apparatus, called an insect "olfactometer" and already mentioned under "olfactory experiments," is now in print (*38*).

The name "olfactometer" means an olfactory measuring apparatus. The one devised has been used to measure or record the responses of insects to attractants and repellents. Briefly stated, it consists of a specially constructed Y-tube, through which insects pass from a dark chamber, being attracted by a light suspended near the free ends of the forks of this tube. The dark chamber is attached to the base of the Y-tube, and a suction apparatus to draw odors through the forks is attached at the point where the base and forks unite. The insects are attracted equally toward the entrances of the forks by the light stimulus, but when ready to enter these forks they are influenced unequally by the odors drawn through the forks, one fork serving as an attractant or repellent side, and the other fork as the control side.

The whole apparatus is so constructed and manipulated that the interfering factors are practically controlled, leaving only the olfactory responses to be recorded.

Only the results obtained with the Colorado potato beetle (*Leptinotarsa decemlineata* Say) will be given here to show that plants attract insects by the odors which they emit. A small potted potato plant was placed in a special chamber of the olfactometer, and a gentle current of air was drawn over it into one fork of the Y-tube by means of the suction apparatus. This supposedly scented air could not be smelled by the writer, but the potato beetles responded to it readily. This experiment on various dates and under different conditions was repeated 24 times, 4 individual tests being considered as one experiment. Each of these 25 experiments plainly showed that the beetles had responded to the odors or emanations from the living potato plants. On an average, 62.7 per cent of them went toward these

odors, while the other 37.3 per cent went (perhaps accidentally) into the control fork. The highest percentage obtained was 76.7 toward the plant odors. Potato beetles, when potato plants are scarce or entirely wanting, feed on the horse nettle, jimson weed, and tomato plant. Experiments similar to the above were conducted in which these plants were used, but as a rule the beetles did not seem to like the odors from these three species. They often failed to respond, and not once did their response give a high percentage of attractiveness.

Eighty experiments were conducted, using the water extracts (diluted juices) and steam distillates of the potato tuber and foliage of the following plants: Potato, jimson weed, horse nettle, tomato, and henbane. In these experiments air was bubbled through the liquids and then drawn into one fork of the Y-tube. In the other or control fork air was bubbled through distilled water. To the odors from the water extracts of potato tubers and potato foliage the beetles always responded, except once, but not as strongly as to the odors from the living potato plants. To the odors from the extracts of the other plants mentioned the beetles usually responded, and more strongly than they had to the odors from the living plants of the same species. To the odors from the steam distillates the beetles generally responded, although many times repellently. The odors from the jimson weed distillate remained repellent throughout the tests; the odors from the other distillates were usually repellent for some time, but later became attractive. The odors from the distillates of the potato tuber, horse neetle, and tomato were only slightly attractive, but those from the potato foliage and henbane were usually strongly attractive. It seems that the steam removed something from these plants which was repellent to the beetles for a time, but later, when this apparently repellent substance had been either changed or volatilized by bubbling air through the liquids, the odors became attractive. When the odors from all these distillates, except from jimson weed, had become attractive to the insects, the liquids gave off a common odor, judged by the nose of the writer. This odor closely resembled that from boiling potatoes or from the juice of freshly cut potatoes, and might be called a potato odor. Since all of these plants belong to the potato family (Solanaceae), it would be interesting to know whether they possess a particular constituent or constituents which emit a common odor, as indicated by the above experiments.

Assuming that the cotton plant emits some volatile and odorous substance which attracts boll weevils from a considerable distance, Power and Chesnut (*44*, *45*), of the Bureau of Chemistry, undertook to isolate this substance. Among the 12 individual substances found by them in the concentrated distillate, which included all the odorous and volatile constituents in the cotton plant, 3 are of particular interest for chemotropic tests. These are the so-called essential oil and 2 basic substances, ammonia and trimethylamine. The percentage of oil isolated is so very small that these writers believe it can not have any significance in attracting boll weevils, and, furthermore, despite the fact that more ammonia was found than trimethylamine, they conclude that if the cotton plant really possessess an attractive odorous constituent, it is in all probability the trimethylamine. If so, the difficult task for the entomologist is to use the substance properly. Of all substances having powerful and persistent odors, trimethylamine is certainly one of them, as illustrated by

the following. Before using a small bottleful of this material, prepared by Power and Chesnut, the writer kept it well stoppered for a few days in a drawer with a small dictionary and some stationery. Both became permeated with the odor, and for weeks afterwards smelled strongly of it. The dictionary still emits a faint odor after a period of two years.

The writer tested the sample of trimethylamine mentioned above in only a preliminary way, using a comparatively few old weevils which had come to the cotton plants from their hibernating places, and the most that he can say in its favor is that it seemed to attract the weevils slightly under certain conditions.

The writer entertains a serious doubt whether it is possible to reproduce accurately the odor or odors which emanate from a plant merely by using the constituents derived from the plant by chemical means. It seems that the most that may be hoped for is an imitation closely resembling the plant odor and even with this it may be difficult and sometimes impossible to deceive the insects. This whole subject is closely related and perhaps analagous to the making of perfumes for the use of the human species. Expert perfumers admit that, after all their long experience in trying to reproduce exactly the odors found in living flowers, the best they have been able to do so far is to imitate closely the natural odors. In many cases the layman can not tell these artificial odors from the natural ones, but the expert perfume tester can readily distinguish them. Insects, which evidently have a keener sense of smell than human beings, should be able to distinguish these differences even more readily.

To find a substance, preferably a sweet one, which would attract boll weevils equally as well as do cotton squares (flower buds), many experiments were conducted in the laboratory and cotton field, using saccharine, sugar, ice-cream powder, a sweetened proprietary calcium arsenate, honey, and three brands of molasses (karo, koo-koo, and blackstrap). The honey and ice-cream powder seemed to attract the weevils slightly, but there was no indication that any of these eight substances can be of practical importance in the control of these insects. Similar results have been obtained by other writers who have tried to attract boll weevils by the use of sweetened baits.

SUMMARY OF SENSES OF COTTON BOLL WEEVIL

Since the sense of smell in boll weevils is believed to be the primary one which aids them in locating cotton plants, the two kinds of so-called olfactory organs have been thoroughly studied and described. The organs, called olfactory pores by the writer, are common to both the adult and larva; but the other so-called olfactory organs, which are nothing more than ordinary innervated hairs, are common only to the antennae of the adult, although similar innervated hairs are also found on other parts of both adult and larva. In the adult the olfactory pores were found on the head capsules, legs, elytra, wings, mouth parts, and at the base of the antennae; in the larva, on the head capsule, base of antennae, mouth parts, clypeus, and second thoracic segment. The individual and sexual variations found in the pores of each of five males and five females were small, although the females have 13.7 per cent more pores than have the males. The anatomy of these pores is like that described for many other beetles by the writer (*30*), except that some

of them are slit shaped, closely resembling those in spiders. These are found on the maxillary palpi of both adult and larva and on the legs of the adult.

The antennae of boll weevils have four types of sense organs, as follows: Many innervated hairs, chiefly on the club; three or four olfactory pores at the base of each antenna; and two so-called auditory organs in the second segment. Not one of these organs, except the olfactory pores (fig. 13), is suited anatomically to receive olfactory stimuli. The hairs are all long, thick-walled throughout their full length, and consequently odors can not pass through them. The so-called auditory organs are totally internal, having no outside connections whatever, so that they certainly can not receive olfactory stimuli.

Innervated hairs were found widely distributed over the surface of the boll weevil, being present on the head capsule, antennae, mouth parts, thorax, legs, wings, abdomen, and genitalia. According to their structure they seem to be fitted only for tactile organs, and, judging from their number and distribution, the sense of touch must be highly developed. When the insect is once upon its proper food and ready to deposit eggs, the tactile sense may be more important than the olfactory sense. The numerous sensitive hairs on the antennae, mouth parts, ovipositor, penis, and elsewhere must certainly render great aid in the various activities of the boll weevil.

No organs were found which can really be called taste organs, although some of the smaller innervated hairs on the mouth parts of both adult and larva would be designated taste organs by certain writers. The present writer believes that the senses of smell and taste in insects are inseparable, but since insects in many cases must first "taste" certain foods before they can discriminate between them, this combined sense may be comparable to ours when we smell flavors.

Three so-called auditory organs were found—two in the pedicel or second antennal segment and one in the base of each wing. We know nothing about the function of these organs, and, indeed, it is difficult to imagine what their offices might be, unless they receive some kinds of waves which we can not detect, such as some of the inaudible sound waves, or even radio waves; but the writer has been informed that radio waves can not be considered in this connection.

Judging from the structure of the compound eyes, the visual field of the boll weevil is small and its image of objects is neither sharp nor distinct, indicating that it can not by sight alone distinguish from a distance cotton plants from certain other plants, or in all probability even the various parts of a cotton plant when it is near them.

As a corollary to the sense organs, the scent-producing organs, sometimes called scent glands or recognition glands, were also studied. These are minute, unicellular, hypodermal glands, and are widely distributed over the surface of the insect. Their secretion comes to the surface, spreads out over the integument, and, besides keeping the body moist, is also supposed to give off an odor which probably serves as a recognition odor among the beetles themselves.

The reader will have noted that the olfactory sense has been greatly emphasized, perhaps too much, but in the present state of our knowledge there seems to be no other tangible sense or senses which serve quite so well to explain how boll weevils find cotton plants from

a distance. When they have once found their food or a place to deposit eggs, we can easily think of two or more senses being employed, in their activities thereafter, touch, smell, and "taste" particularly being used during feeding and egg laying, as has recently been pointed out by Richardson (*49*), who reviewed the literature pertaining to the oviposition response of insects.

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EFFORTS TO DETERMINE THE MEANS BY WHICH THE COTTON-WILT FUNGUS, FUSARIUM VASINFECTUM, INDUCES WILTING[1]

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HISTORICAL REVIEW

In 1899 Smith (*25*)[2] suggested that the wilting of cotton, cowpea, and watermelon plants, which were infected with closely related fungi of the genus Fusarium, was due to a mechanical plugging up of the vascular system. He was able to find considerable mycelium within the water-conducting tubes, and assumed that the amount present was sufficient to act as an obstruction to the passage of liquid upwards, thus causing the condition known as "wilt." His explanation has for many years been the one commonly accepted by writers of various well-known texts, as well as by authors of special bulletins dealing with cotton wilt, including Duggar (*7*), Stevens (*26*), and Orton (*20, 21, 22*).

As this theory has within recent years been questioned in a number of comparable diseases caused by species of Fusarium, such as those causing potato wilt (*3; 5, p. 270; 11; 12*) and tomato wilt (*9, p. 75*), as well as wilts induced by other fungi and bacteria (*2, 8, 23*), it seemed desirable to ascertain the mode of action of *F. vasinfectum* in its method of inducing cotton plants to wilt. It appeared especially worth while to undertake such a study, in view of the lack of wilting noticed in certain infested fields at times, which in other seasons showed a high wilt percentage. Was this explainable on the basis of difference in environmental conditions, such as soil or air temperatures, soil moisture, etc., or was it to be regarded as a difference in the degree of plugging up of the vascular systems resulting from a difference in the amount of growth of the fungus? If wilting is simply a response to a mechanical obstruction, then it is quite conceivable that any condition which would favor the growth of the fungus, irrespective of its effect on the host, should make for a wilting of the plant; and, on the other hand, any condition which would be unfavorable to the growth of the fungus should make for wilt-free plants.

One of the first to experiment on the factors involved in the wilting of a plant attacked by a microorganism was Hutchinson (*14*) of India. In 1913, working on a disease of tobacco which he considered to be caused by *Bacillus solanacearum*, he fed plants a substance which had been precipitated by alcohol from pure bouillon cultures of the pathogene. The plants which obtained this substance developed wilt, while those fed with a boiled solution of the same substance did

[1] Received for publication Aug. 19, 1926; issued January, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 1161.

not wilt. He considers the wilting as due to the interference of osmotic pressure consequent on protoplasmic intoxication, and in the advanced stages the water supply is interfered with by the formation and acceleration of gum masses in vessels. The actual factor which brought about these conditions is a thermolabile, toxic substance.

Coons (*5, p. 270*) reporting on the work of Goss in 1916 in a preliminary fashion, calls attention to the wilting of Irish potato plants when placed in a filtrate representing a synthetic solution upon which a species of Fusarium had been grown. Control plants placed in an uninoculated medium remained healthy. It was concluded that wilting was due to poisons generated by the fungus.

Haskell (*12*) in 1919 came to a similar conclusion concerning wilt of Irish potatoes caused by *Fusarium oxysporum*. He injected solutions of oxalic and salicylic acids, as well as liquid culture media upon which the fungus had been grown, and obtained a browning and necrosis of the vascular systems with all. By making microscopic observations of stems of infected plants, he "found no instance of a stoppage of the trachea sufficient to shut off the passage of sap."

In 1919 Bisby (*3*) reported filtering several species of fungi from the liquid nutrient media upon which they had grown, and placing leaves of different plants into the filtrates. The fungi used were: *Fusarium oxysporum*, *F. discolor sulphureum*, Fusarium from pea, Rhizoctonia, and Penicillium. Wilting occurred within a few hours with some extracts, a fact which he feels is not to be explained by changes in acidity. Excised leaves placed in uninoculated media and in water did not wilt until considerable time had elapsed. The injurious effect was also noted after boiling the filtrates and after diluting them considerably. He concludes that the wilting is not due to some poison which is specifically active against certain plants, since "potato leaves wilt as readily in old Rhizopus solutions as in solutions in which *F. oxysporum* had grown."

Brandes (*4*) in 1919 found that cultures of *Fusarium cubense* grown in Richards' solution when filtered free from the fungus produced a marked wilting of buckwheat and bean plants, as well as banana leaves. He obtained the same results, using Uschinsky's solution as a medium, and concluded that in the disease known as banana wilt, wilting is not due to plugging of the vessels by mycelium, but is probably the result of toxic excretions by the fungus.

Young and Bennett in 1920 (*28*) made a further effort to determine the method by which *Fusarium oxysporum* induces wilting of potato plants. They found that filtrates of this fungus growing upon Richards' solution were increasingly toxic to potato plants as the age of the cultures increased up to the fortieth day. Furthermore, with this increase in toxicity there is noted, after a 10-day period, a gradual decrease in the hydrogen-ion concentration, reaching P_H 7.4 on the fortieth day from an initial reaction of P_H 5.0. But upon uninoculated solution adjusted to P_H 7.4, there is no toxicity noted when cut stems are placed in it. From this it is concluded that the change in reaction, while not the primary cause of wilting, indicates the presence of a compound with a slightly alkaline reaction. Autoclaving or boiling did not alter the toxicity of this substance.

In 1921 Ajrekar and Bal (*1*) of India, working on cotton wilt and using Hutchinson's (*14*) method, previously mentioned, were unable to obtain wilting of cotton plants by means of the alcoholic extract. They do not, however, conclude from their very meager tests (only two plants were used) that no toxins are produced by the fungus; on the contrary, when they sectioned diseased plants and examined them microscopically, the number of vessels which were not occupied by the fungus was so great in comparison with the number occupied that they suspected the action of a toxic substance rather than a mechanical plugging up by fungal mycelium.

Bewley (*2*) in 1922 found that the liquid cultures in which *Verticillium albo-atrum* was grown (the fungus causing "sleepy disease" of tomato) contained a substance capable of causing wilt when filtered free from the fungus. He attempted to isolate the enzymes produced by the fungus during growth and decided that no endoenzymes capable of causing wilt were present. He concluded that the wilting was caused by an exoenzyme which he precipitated with alcohol, and which he found to be greatly reduced in activity by heating to 100° C.

Picado (*23*) in 1923 attempted to determine the nature of the substances secreted or excreted by parasitic fungi in which the host tissues are either injured or killed at some distance from the point of attack. He worked with *Verticillium albo-atrum*, *V. duboys*, both parasites of the potato; *Fusarium solani* from the melon; *F. cubense*, the pathogene of banana wilt; and with a saprophytic Penicillium. The results indicated that the withering of the plants experimented upon, the browning of their vessels, and the destruction of their tissues were not all produced by the same causes, though in each instance evidence was seen of the action of substances having toxic properties, such action resulting in the symptoms produced in the different diseases. Picado draws a distinction between true toxins or enzymes and substances having poisonous properties. After extracting the albuminoids, enzymes, and toxins from the mycelium with alcohol and concentrating the liquid from the crushed mycelium, he obtained a waxy, brownish residue. This proved to be thermostable and very toxic to plants. The heated extracts were considerably more potent in producing wilt than the endoenzymes and endotoxins. Picado's conclusion is that the poisons produced by these fungi are not true toxins in the sense of toxins against which immunization is possible. They may be compared in action to poisons found in some mushrooms such as "Amanita-toxin," and he suggests that the action at a distance of certain parasitic fungi may be due to a chronic poisoning of the plant.

In 1923 Fahmy (*8*) described his efforts to determine the cause of wilting brought about by *Fusarium solani*. The fungus was grown in pure culture in Richards' solution, and then filtered under sterile conditions. He found that the unboiled as well as the boiled filtrate possessed marked toxic properties, and he determined the presence in the filtrate of ammonia as well as oxalic acid. But the amounts present did not seem to him to be sufficient to account for the wilting. He also found that the substance responsible for wilting was thermostable and nonvolatile.

Goss (*11*) in 1924 concluded upon the examination of infected potato plants that the vascular elements were not blocked by the growth of the fungus, *Fusarium eumartii*, and that the tissues were often killed in advance of the organism. He grew the fungus on a slightly modified Richards' solution, and after filtering he found the filtrate capable of wilting plants in a relatively short time. The boiled extract, however, produced no wilting. In contrast to this, filtrates of another wilt-inducing fungus, *Fusarium oxysporum*, remained toxic after boiling.

In the biennial report of the director of the Kansas Agricultural Experiment Station, which appeared in 1924 (*9, p. 75*), it is recorded that *Fusarium lycopersici* has been found to secrete an enzyme which, when precipitated, dried, and redissolved in distilled water, causes a rapid wilting of young plants when their cut stems are immersed in the solution. Boiling destroys the toxicity of the enzyme. Both the residue and the dialyzate of nutrient solutions upon which the fungus was grown produced wilting of young plants. From this it is concluded that two toxic substances exist, one a colloid and the other a crystalloid.

The recent work of Johnson and Murwin (*15*) on the toxic substance produced by the bacterium causing wildfire of tobacco is of interest. They noticed marked yellowish halos and chlorosis of bud leaves in the absence of organisms or with a few present. They concluded that a soluble, toxic substance is formed by the organism. When it was cultured on potato agar and a suspension made of the pathogene in water, which was filtered through a Berkefeld filter, the application of the cell-free filtrate on tobacco leaves resulted in the production of typical halos.

Very recently Jochinai (*27*) concluded that the rapid wilting of flax plants attacked by *Fusarium lini* was due to the obstruction of the xylem consequent upon the production of gas, while the poisoning of the cells was caused by alkalinity produced by the fungus. His conclusions are based entirely upon *in vitro* studies of fungal cultures and no effort was made to test these theories in healthy host plants.

These references, and others not cited, all indicate that in a number of diseases, many of them comparable to the wilt of cotton in symptoms as well as in causal agent, the theory of a mechanical plugging by the fungus causing wilt has been discarded and in place of it there is proposed the action of one or more toxic substances given off by the various fungi. These toxic substances are directly or indirectly responsible for wilting and other pathological phenomena. But it is quite obvious that in very few instances has a careful study been made of the nature of the poisonous principles produced by the fungi. It has also been revealed that an attempt to prove the production of a toxic substance by the cotton wilt fungus resulted in failure.

EXPERIMENTS TO DETERMINE THE ACTION OF FILTRATES ON COTTON PLANTS

From the literature cited it appears that the common method used in proving the presence or absence of toxic substances is to grow the pathogene on some nutrient medium—frequently Richards'

solution is utilized—filter the organism from the medium, and place the plants, usually with their roots cut off, into the filtrate. This method has been utilized by the writer (its adequacy will be discussed later). Table 1 presents in brief the number of such tests, and the results obtained. Unless otherwise noted, the plants consisted of vigorouly growing seedlings which were carefully removed from the pots, then placed in water and the roots cut off under the water. The cultures of the fungi were grown at room temperature for two to three weeks in Erlenmeyer flasks containing about 300 c. c. of medium.

TABLE 1.—*Action of various filtrates on cotton plants*

Experiment No.	Date	Fungus	Strain	Medium	Type of filtration	Number of plants treated	Number of plants wilted	Number of plants free from wilt	Length of time for wilt to appear
	1925								
1a	Feb. 16	F. vasinfectum	1924 Terrace	Nutrient broth	Fine filter paper	12	0	12	
				Richards' solution	do	12	12	0	18 hours.[a]
1b	do	do	do	Uninoculated Richards' solution		12	0	12	
1c	do			Richards' solution	Fine filter paper	12	[b]4	8	20 hours.[b]
1d	do	F. lycopersici			do	6	6	0	15 hours.
2a	Feb. 24	F. vasinfectum	1924 Terrace	do	Porcelain filter	6	5	1	Do.
2b	do	do	do	do		12	0	12	
2c	do			Uninoculated Richards' solution		3	2	1	
2d	do	F. vasinfectum	1924 Terrace	Richards' solution, acidulated	Fine filter paper	6	6	0	24 hours.
3a	Feb. 25	do	do	Richards' solution	do	6	6	0	Do.
3b	do	do	do	do	Porcelain filter	8	0	8	
4a	Mar. 3	do	do	Uschinsky's solution	Berkefeld N filter	8	0	8	
4b	do			Uninoculated Uschinsky's solution.					
5a	Mar. 16	F. vasinfectum	1924 Acala	Richards' solution	Berkefeld N filter	3	3	0	10 minutes.
5b	do			Tap water		3	0	3	
6a	Mar. 27	F. vasinfectum	1924 Acala	Richards' solution	Berkefeld N Filter	4	4	0	20 hours.
6b	do			Uninoculated Richards' solution.		4	0	4	
6c	do	F. vasinfectum	1924 Acala	Richards' solution diluted with equal quantity of distilled H_2O.	Berkefeld N filter	4	4	0	20 hours.
6d	do			Uninoculated Richards' solution diluted as in 6c.		4	0	4	
6e	do	F. vasinfectum	1924 Acala	Richards' solution diluted with distilled H_2O, 1:2.		3	0	3	
7a	do	do	do	Richards' solution containing glucose.	Berkefeld N filter	5	5	0	20 hours.
7b	do			Uninoculated Richards' solution.		5	5	0	Do.
7c	do	F. vasinfectum	1924 Acala	Richards' solution diluted with equal quantity H_2O.	Berkefeld N filter	5	5	0	Do.
7d	do			Uninoculated Richards' solution diluted as in 7c.		5	5	0	Do.
8a	Mar. 31	F. vasinfectum	1924 Acala	Richards' solution	Berkefeld N filter	3	3	0	5 hours.
8b	do	do	do	do	Filtrate of 8a diluted with equal quantity H_2O.	3	3	0	Do.
8c	do	do	do	do	Filtrate of 8a diluted with twice its volume of H_2O.	3	0	3	
8d	do			Uninoculated Richards' solution.		3	0	3	

8e	do			8d solution diluted with equal volume H_2O.		3	0	3	
8f	do			8d solution diluted with twice its volume of H_2O.		3	0	3	
9a	do	F. vasinfectum	1924 Acala	Richards' solution distilled at 100° C.	Berkefeld N filter	4	4	0	24 hours.
9b	do	do	do	Richards' solution distilled at 110–120° C.	do	4	0	4	
9c	do	do	do	Residue of distillation in 9a and 9b, diluted 1 to 5 parts H_2O.	do	4	4	0	8 hours.
9d	do	do	do	Same as 9c, diluted 1 to 20 parts H_2O.	do	3	3	0	10 hours.
9e	do	do	do	Same as 9c, diluted with H_2O to make a 2 per cent solution.	do	5	5	0	24 hours, slight wilting.
9f	do	do	do	Same as 9c, diluted to make a 1 per cent solution.	do	4	4	0	48 hours, slight wilting.
10a	June 24	do	Single spore	Richards' solution	do	9	5		5 hours.
10b	do	do	do	Richards' solution (boiled)	Berkefeld N filter	9	5		Do.
10c	do			Uninoculated Richards' solution.		9	0	9	
	1926								
11a	Jan. 11	F. vasinfectum	1925 isolation	Richards' solution	Berkefeld N filter	3	3	0	5 hours.
11b	do	F. tracheiphilum		do	do	3	3	0	Do.
11c	do			Uninoculated Richards' solution.		3	0	3	
11d	do			Tap water		3	0	3	
12a	Feb. 5	F. vasinfectum	1925 isolation	Richards' solution	Fine filter paper, filtrate distilled.	4	4	0	16 hours.
12b	do	do	do	do	Distillate of 12a diluted with 4 parts H_2O.	4	[b] 4	0	Do.
12c	do	do	do	do	Residue of 12a	4	4	0	Do.
12d	do	do	do	do	Residue of 12a diluted with 4 parts H_2O.	4	4	0	Do.
12e	do			Uninoculated Richards' solution diluted with 4 parts H_2O.		4	0	4	
12f	do			Tap water		4	0	4	

[a] Plants kept in the shade, temperature about 22° C.
[b] Slight wilting.
[c] After standing in the filtrate for 20 hours the plants of experiments 1b, 1c, and 1d were placed in tap water to prevent the action of any contaminating organisms that might appear in the filtrate.

EXPERIMENTS 1A TO 1D

At the end of a week the plants originally placed in uninoculated Richards' solution appeared perfectly healthy; those in the *Fusarium vasinfectum* filtrate were dead; those in *F. lycopersici* filtrate, with the exception of two plants that appeared normal, showed varying degrees of withering and flagging. On the upper parts of the stems of plants treated with *F. vasinfectum* filtrate, profuse growth of a Fusarium developed, but the plants subjected to the *F. lycopersici* filtrate showed no such growths. As will be shown later, filtering through fine filter paper does not remove all of the fungus, and a probable explanation for the lack of fungal development on wilted stems of plants treated with *F. lycopersici* filtrate is that that fungus will not

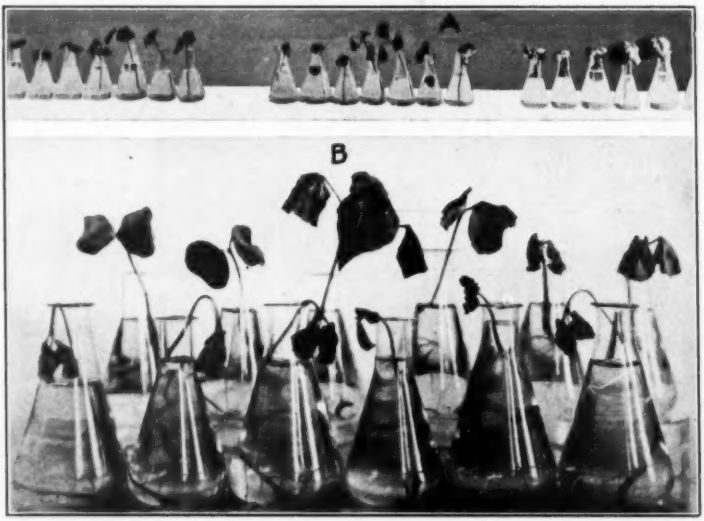

FIG. 1.—A, comparison of the action of a filtrate of *Fusarium vasinfectum* (the 7 plants in the center), a filtrate of *F. lycopersici* (the 7 plants on the left), and uninoculated Richards' solution (the 6 plants on the right). Photographed at the end of 24 hours' treatment. B, comparison of the action of a filtrate which had passed through a bacteriological porcelain filter (top row) and a filtrate which had passed through a fine grade of filter paper (bottom row). Photographed at the end of 24 hours' treatment. In both cases the plants had been carefully removed from the soil and the roots immersed in the filtrate

grow on cotton, although the filtrate contains ingredients which are poisonous. The interiors of wilted plants all showed brownish discoloration of the xylem elements, while the unwilted control plants remained normal.

One of the interesting facts noted in this series of experiments is the difference between the action of the nutrient-broth filtrate and Richards' solution (see fig. 1), both representing transfers from the same fungal culture, and both of exactly the same age. The fact that the broth filtrate possessed no toxic properties, while Richards' solution proved markedly toxic, may explain the reason for the failure of Ajrekar and Bal (*1*) to obtain toxic action of the cotton-wilt fungus when grown in peptone bouillon. It also brings to mind the marked

difference in physiological processes which are apt to appear with the use of different types of media, or under other differences in environmental conditions. For example, it is well known that in the presence of certain organic nitrogen compounds the metabolic products attending the growth of numerous microorganisms are apt to be different from those found when inorganic nitrogen is supplied. Again, in the presence of certain carbohydrates, acids and gas are formed, while in the absence of these no acid or gas formation takes place. It opens up the whole question as to the significance to be attached to findings of toxic properties with any medium which does not closely approach the chemical and physical make-up of the natural host.

EXPERIMENTS 2A TO 2D

The porcelain filter used in experiments 2a to 2d was one which did not permit the passage of a 10 per cent solution of defibrinated ox-blood hemoglobin. It had been previously autoclaved for 30 minutes at 15 pounds pressure, and the whole process of filtering carried on under aseptic conditions. Platings made of this filtrate remained sterile.

The one plant which had not wilted in the 15-hour period in experiment 2b gradually succumbed, so that at the end of 36 hours all of the plants treated with the filtrate which had passed through the porcelain filter appeared just as withered and sickly as those which had been placed in the filtrate of the filter paper. Platings made of the latter showed that a good many viable spores of the fungus had passed through the pores of the paper. These made typical growth, with the pinkish color characteristic of *Fusarium vasinfectum* when grown on potato-glucose agar.

As the uninoculated Richards' solution tested P_H 4.2, while the filtrate of the 3-weeks-old culture tested P_H 6.6, it seemed worth while to determine if the change in acidity had any influence on the toxic properties of the filtrate. The filtrate in experiment 2d was acidulated with hydrochloric acid until it tested P_H 4.4. As far as this limited test goes, it appears that the change in hydrogen-ion concentration in itself was not responsible for the poisonous nature of the filtrate.

EXPERIMENTS 3A AND 3B

Experiments 3a and 3b were designed primarily to test the action of the filtrates upon uncut stems. The plants were carefully removed from the pots of soil in such a manner as to disturb the root systems as little as possible. The roots were washed free of soil and then placed in the filtrates. The results show that the toxic substance can be absorbed by the roots and produce typical wilt symptoms. May not this fact explain why in a given field certain plants exhibit at times a stunted and partially wilted effect, without any evidence of infection, while other plants readily yield cultures of the cotton-wilt fungus?

One fact observed in this experiment was the more rapid and complete wilting of the plants placed in the filter-paper filtrate, as compared with those placed in the filtrate which had passed through the porcelain cylinder. This had also been noticed in the previous experiments. It has already been noted that solutions which passed through

fine filter paper still harbored the fungus. This unquestionably would also be true of cotton filters, and as a number of investigators whose work has been previously reported say nothing of the use of bacteriological filters, it opens up the question as to their claims of toxic substances. Lutz's (*19*) claim that poisons are partially withheld when clay filters are used is certainly not true of a number of microorganisms, as shown, for example, by Young and Bennett (*28*).

In order to test this point further, isolations were attempted from the wilted plants of both types of filtrates. The stems of wilted plants were cut into pieces about 1.5 cm. long, the surfaces sterilized with mercuric chloride, washed in sterile water, and placed in Petri dishes containing potato-glucose agar. Out of six pieces derived from plants subjected to the filter-paper filtrate, three developed typical growth of *Fusarium vasinfectum*, while none of the pieces representing plants subjected to the filtrate which had passed through the porcelain filter showed the presence of any type of microorganism. It is not intended to suggest that cotton or filter-paper filtrates which possess poisonous properties owe their action primarily to the presence of the pathogene, but it is suggested that unless the pathogene is completely removed there is no good evidence for the toxicity theory.

EXPERIMENTS 4A AND 4B

The formula used for the Uschinsky's solution is that given in Giltner's "Microbiology," (*10*) under the heading "Uschinsky's asparagin medium." (There are a number of solutions going under the name of Uschinsky's solution, but as far as the writer can judge, their main point of similarity is in the name.) The test was designed to obtain further information on the difference to be expected in the toxic properties of a filtrate representing different types of media. As in nutrient-broth filtrate, there appear to be no toxic properties developed in the Uschinsky solution. It should be noted, however, that although cultures were 25 days old, growth was not as abundant as in Richards' solution. The marked sweetish odor present in the latter, as well as the amber-brown color, were entirely absent in the asparagine medium. It is also to be noted that while Richards' solution develops a hydrogen-ion concentration of around P_H 6.6, the Uschinsky solution develops marked alkalinity, P_H 8.4, in the same time interval. Probably this is due to the use of organic nitrogen in the latter medium. The difference in hydrogen-ion concentration is shown below.

Type of solution	H-ion concentration
Uninoculated Richards'	P_H 4. 2
Richards' with 2 weeks' growth of *Fusarium vasinfectum*	P_H 6. 6
Uninoculated nutrient broth	P_H 6. 8
Broth with 2 weeks' growth of *F. vasinfectum*	P_H 7. 4
Uninoculated Uschinsky's solution	P_H 4. 8
Uschinsky's solution with 25 days' growth of *F. vasinfectum*	P_H 8. 4

EXPERIMENTS 5A AND 5B

As a marked difference had been noted in the behavior of different strains of the cotton-wilt fungus, it seemed worth while to test the effect of a different strain from that previously described and listed as 1924 Terrace. The strain here used behaved quite differently. Wilting occurred within a few minutes, but the very bright sunlight,

as well as the high temperature present in the greenhouse at the time, may have had something to do with this, and the control (plants placed in tap water) was not considered adequate. Unfortunately, there was no uninoculated solution available at the time. Under the conditions cited above, it is quite possible that rapid evaporation may have so concentrated the filtrate as to cause wilting irrespective of any toxic ingredients. The writer suspects that the same thing occurred in a number of instances in which the investigator claims wilting in a very short period. The filtrate here discussed represented a 15-day-old culture with a marked sweetish odor, suggesting somewhat the odor of lactic acid to the writer but not to others. It had a hydrogen-ion concentration similar to the uninoculated solution, P_H 4.4, in marked contrast to the P_H 6.6 developed in the 2-weeks-old culture of the 1924 Terrace strain. Tests with ether-ferric chloride as described in Hawk's "Physiological Chemistry" (*13*) for lactic acid, developed a slightly greenish tinge, indicating the presence of lactic acid; however, the markedly brownish color of the medium prevented an absolute diagnosis. In any case, the quantity of lactic acid present, if any, was probably so small as to be of no particular significance.

EXPERIMENTS 6A TO 6B

The object of this group of experiments was to determine the power of the filtrate to induce wilting when it is diluted. Obviously a solution upon which a fungus has been growing for three weeks has lost part of its nutrients as well as considerable water. Also, as Brandes (*4*) has pointed out, there is the possibility of a greater osmotic pressure being developed in a Richards' solution with sucrose as a source of carbon because of the conversion of the disaccharide to two monosaccharides. Plants placed in the filtrate diluted with an equal quantity of distilled water showed a slight wilting in the same time interval as those placed in the undiluted filtrate, clearly indicating that it is not the possible increase in osmotic pressure that is responsible for the toxic action. On the other hand, the filtrate, diluted with twice its volume of water, possessed no appreciable toxic action.

EXPERIMENTS 7A TO 7D

It seemed worth while to determine the toxic action of a filtrate which had for its supply of carbon a monosaccharide. For this purpose chemically pure dextrose (anhydrous dextrose obtained from a commercial company) was substituted for the sucrose in the Richards' solution at the same concentration, 5 gm. in each 100 c. c. of solution. The fungus seemed to grow as well on this medium as on the sucrose medium, although no effort was made to get weights of dry fungal mats. But for some reason the uninoculated glucose medium induced a wilting, especially of the cotyledons. However, as the time of observation was increased, a difference could be clearly seen in the amount of wilting. At the end of 66 hours all the plants kept in the concentrated filtrate showed a marked wilting of the true leaves, as well as of the cotyledons; those kept in the concentrated and uninoculated Richards' solution showed only a wilting of the cotyledons; while the diluted filtrate as well as the diluted uninoculated solution showed a comparable difference. The wilting

was not as great in the diluted filtrate as in the concentrated filtrate. It appears that the filtrate possessed some toxic properties not present in the original medium, but the disadvantage of using dextrose instead of sucrose in Richards' solution appears obvious from this experiment. The hydrogen-ion concentrations of the solutions here mentioned were as follows (1924 Acala strain, 3-weeks-old cultures):

Type of solution	H-ion concentration
Uninoculated Richards' solution with dextrose	P_H 4. 0
Uninoculated Richards' solution with sucrose	P_H 4. 4
Three-weeks-old culture on dextrose-Richards' solution	P_H 6. 8
Three-weeks-old culture on sucrose-Richards' solution	P_H 5. 0

A comparison of these data with the hydrogen-ion data previously given indicates that at the end of the same time interval there is a noticeable difference between the hydrogen-ion concentration in this strain of *Fusarium vasinfectum* and the other strain.

EXPERIMENTS 8A TO 8F

Livingston (*18*) has shown that sunlight undoubtedly influences the rate of transpiration, and it appeared desirable to test its action on the rate of wilting. This series of experiments apparently brings out quite clearly the fact that very bright sunlight affects the rate of wilting, for in one filtrate exposed to strong sunlight plants wilted in approximately one-fourth the time required by plants in a similar filtrate in diffused light. The explanation would appear to be as follows: As transpiration increases, there is a greater intake of water and probably of the dissolved substances, and this increase would account for the more rapid wilting. That it is not due merely to a concentration of the filtrate outside of the plant appears to be evident from the fact that control plants in the uninoculated solution do not wilt under the same conditions in the same length of time.

EXPERIMENTS 9A TO 9F

This series of experiments was designed to help in determining the nature of the toxic substance. Was it volatile, and was it thermolabile? About 300 c. c. of the filtrate which had passed through a Berkefeld filter was subjected to distillation, for the first 30 minutes at about 100° C., and later at 110° to 120°. The first distillate was collected in about 100 c. c. of cold distilled water, the point of the condenser resting below the water level so as to prevent the escape of any gas. The odor of the first distillate was very sharp, pungent, and somewhat sweetish. Tests for amines with gold chloride and with chloroplatinic acid were negative. The odor of the second distillate was not as distinct as the first. The residue, consisting of 75 c. c., contrasted with the original 300 c. c., had a dark-brown color and a strong caramellike odor. Plants placed in the first distillate wilted within 24 hours, while control plants placed in uninoculated Richards' solution remained turgid. In contrast to this, the plants placed in the second distillate showed no wilting in the same period. Evidently the volatile substance possessing toxic properties passed off quickly and completely. However, neither of the distillates possessed the extreme toxicity of the residue. When this was diluted with five parts of water, the plants subjected to it showed signs of flagging within several hours, in spite of the fact that the dilution was

more than sufficient to make up the original volume. Even the residue diluted with 20 times its volume of water showed toxic qualities almost equal in severity to those of the lesser dilution. Dilutions making a 2 per cent and 1 per cent solution of residue possessed but slight toxic properties. Compared to these, the first distillate appeared more toxic. From this experiment one may conclude as follows: There are two toxic substances present in the filtrate, one volatile and possessing slight toxic properties and the other nonvolatile, thermostable, and increasing in toxicity upon heating. Tests for amines were undertaken because a number of these are known to be highly toxic, some of them resulting from the action of microorganisms. The interesting work of Krueger and Alsberg (*16*) in detecting mono-, di-, and triamines in cultures of *Pseudomonas solanacearum*, a wilt producer, also indicated possibilities for their detection in the media sustaining the cotton-wilt fungus. While the tests previously cited are commonly used for detecting amines, including the closed ring ones, the alkaloids, the writer does not feel that the negative results obtained are conclusive. The detection of various amines in the filtrate of *P. solanacearum* does not necessarily mean that these substances have anything to do with wilting, and Krueger and Alsberg make no such claim. The fact is that amines are not known to have any definite physiological function in plants, including the microorganisms, and are found constantly in only a small proportion of them.

EXPERIMENTS 10A TO 10C

It seemed worth while to test a third strain of the cotton-wilt fungus since the two others had shown such divergence in color developed in the media and in the hydrogen-ion concentration. A 3-weeks-old culture tested P_H 6.2, while the uninoculated control of the same age tested P_H 4.0. In all respects the filtrate closely resembled the 1924 Terrace strain. Krueger in correspondence had suggested testing for nitrites in the filtrate. Such tests are common in bacteriological work, but rather rare in work with fungi. Using the sulphanilic acid-alpha naphthalamine method, a very positive test for nitrites was obtained in the filtrate, while the uninoculated Richards' solution gave entirely negative results with no indication of a pink color. The test was repeated several times and with the same result. There is no doubt that in a medium containing nitrate the fungus is capable of reducing this to nitrites. Furthermore, it was interesting to see that a 3-weeks-old culture of the same strain, growing upon Uschinsky's solution, gave only negative results when tested for nitrites. It will be recalled that with the latter medium the filtrate is not toxic to cotton and that the medium itself contains no nitrates.

Of the 9 plants placed in each of the different solutions represented in experiments 10a, 10b, and 10c, 5 were about 10 inches high and the 4 remaining were seedlings. Was there any difference in toxicity to be noticed in older plants compared to seedlings? The results show that while the seedlings wilted completely within five hours in the unboiled filtrate and were quite flaccid in the boiled filtrate, the larger plants, with the exception of one in each, showed no such ill effects. The larger plants were not as thrifty as the seedlings,

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more than sufficient to make up the original volume. Even the residue diluted with 20 times its volume of water showed toxic qualities almost equal in severity to those of the lesser dilution. Dilutions making a 2 per cent and 1 per cent solution of residue possessed but slight toxic properties. Compared to these, the first distillate appeared more toxic. From this experiment one may conclude as follows: There are two toxic substances present in the filtrate, one volatile and possessing slight toxic properties and the other nonvolatile, thermostable, and increasing in toxicity upon heating. Tests for amines were undertaken because a number of these are known to be highly toxic, some of them resulting from the action of microorganisms. The interesting work of Krueger and Alsberg (*16*) in detecting mono-, di-, and triamines in cultures of *Pseudomonas solanacearum*, a wilt producer, also indicated possibilities for their detection in the media sustaining the cotton-wilt fungus. While the tests previously cited are commonly used for detecting amines, including the closed ring ones, the alkaloids, the writer does not feel that the negative results obtained are conclusive. The detection of various amines in the filtrate of *P. solanacearum* does not necessarily mean that these substances have anything to do with wilting, and Krueger and Alsberg make no such claim. The fact is that amines are not known to have any definite physiological function in plants, including the microorganisms, and are found constantly in only a small proportion of them.

EXPERIMENTS 10A TO 10C

It seemed worth while to test a third strain of the cotton-wilt fungus since the two others had shown such divergence in color developed in the media and in the hydrogen-ion concentration. A 3-weeks-old culture tested P_H 6.2, while the uninoculated control of the same age tested P_H 4.0. In all respects the filtrate closely resembled the 1924 Terrace strain. Krueger in correspondence had suggested testing for nitrites in the filtrate. Such tests are common in bacteriological work, but rather rare in work with fungi. Using the sulphanilic acid-alpha naphthalamine method, a very positive test for nitrites was obtained in the filtrate, while the uninoculated Richards' solution gave entirely negative results with no indication of a pink color. The test was repeated several times and with the same result. There is no doubt that in a medium containing nitrate the fungus is capable of reducing this to nitrites. Furthermore, it was interesting to see that a 3-weeks-old culture of the same strain, growing upon Uschinsky's solution, gave only negative results when tested for nitrites. It will be recalled that with the latter medium the filtrate is not toxic to cotton and that the medium itself contains no nitrates.

Of the 9 plants placed in each of the different solutions represented in experiments 10a, 10b, and 10c, 5 were about 10 inches high and the 4 remaining were seedlings. Was there any difference in toxicity to be noticed in older plants compared to seedlings? The results show that while the seedlings wilted completely within five hours in the unboiled filtrate and were quite flaccid in the boiled filtrate, the larger plants, with the exception of one in each, showed no such ill effects. The larger plants were not as thrifty as the seedlings,

and this fact may have influenced the results. Eventually the larger plants also wilted, but whether this was due to the filtrate or to some secondary organism introduced into the filtrate was not determined. It should be noted that bacteria of various sorts thrive very well on the filtrate when they have considerable difficulty in growing in the uninoculated solution. The reason doubtless is to be found in the hydrogen-ion concentrations, considerable acidity, P_H 4.0, of the uninoculated medium acting as the deterrent. The control plants in the uninoculated medium remained turgid and healthy looking for about 48 hours. Irrespective of poisons, the writer finds that it is more difficult to keep plants thrifty in water solutions during the warm parts of the year than during the cooler parts. As has already been shown, the boiled filtrate also possessed toxic properties. The filtrate in a cotton-stoppered Erlenmeyer flask was heated over a water bath for about 20 minutes at 100° C. However, the amount of flaccidity and wilting in the five-hour period was not as great in the boiled filtrate as in the unboiled, indicating that the heating may have driven off some of the volatile toxic substance. Boiling, as reported elsewhere, does not destroy the toxic principles.

EXPERIMENTS 11A TO 11D

It has already been recorded that filtrates of the tomato-wilt fungus, *Fusarium lycopersici*, exercise but slight toxic effects upon cotton plants. Would the filtrate from a fungus that is more closely related to *F. vasinfectum* be more toxic? The cowpea-wilt fungus, *F. tracheiphilum*, is so closely related to the cotton-wilt fungus that for a long time they were considered as one species. Indeed, the main difference known at present is the difference in susceptible hosts. This experiment indicates that the filtrates of the two possess about the same toxicity to cotton. This might be interpreted to mean that studies on filtrates are not conclusive for toxicity study, inasmuch as they are not specific, as Lutz (*19*) and Bisby (*3*) have pointed out. There is, however, another possible explanation. When one severs the root system of a plant, or uses an excised leaf as Bisby did, any selective action is of course largely destroyed for that plant. But irrespective of this, we are dealing here with parasites that push their way into and grow rather profusely within a susceptible plant. In spite of the fact that the group of Fusaria as a whole are remarkable in their range and frequency, easily living on almost all kinds of dead matter, they show marked restrictions in their capacity to parasitize, often limiting themselves to but one species or only to certain varieties of that species, and then only attacking under certain restricted conditions. In other words, the possibility of filtrates of various and diverse fungi causing wilting of a given plant does not necessarily overthrow the experimental data obtained in filtrate studies of the parasite specific to that plant. One must first show that a certain fungus is capable of penetrating and living within a given plant before toxicity studies of its filtrates are rejected or accepted.

EXPERIMENTS 12A TO 12F

These experiments were undertaken to find out more about the substances in the filtrate of Richards' solution which possessed toxic properties. As the filtrate was to be heated, the fungus was filtered

Insert as second paragraph under "Experiments 11A to 11D" (page 1156)

C. R. Hursh (Sur la toxicité des milieux de cultures des champignons phytopathogènes vis-à-vis des plantes. Rev. Path. Vég. et Ent. Agr. *12:* 137–141. 1925) has concluded that too much importance may be attached to the phenomenon of wilting in plants placed in filtrates of diverse fungi. He found that wilting disappeared when the plants were transferred from the filtrate to fresh water and that these same plants when replaced in the filtrate were observed to wilt either much more slowly or not at all. His very meager experimental data, however, may be questioned. (See, for example, the account of Experiment 1B in which plants left in the filtrate of *F. vasinfectum* for 20 hours and then transferred to tap water, instead of recovering, were dead at the end of a week.)

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off by means of fine filter paper. In spite of the fact that the solution was markedly less acid, judged by the hydrogen-ion concentration, than the uninoculated solution, yet there was the possibility of the formation of organic acid having a low dissociation constant, together with other metabolic products possessing alkaline properties. At the suggestion of A. P. Krueger, strictly chemically pure calcium carbonate was added to the filtrate, together with a few drops of toluol. After standing 48 hours, the carbonate was filtered off and the filtrate was concentrated by heating and distilling in vacuo. Krueger had suggested that such a concentrated filtrate might in time develop crystals representing calcium salts of any organic acids which might be present. At the same time, it seemed desirable to gather the distillates in order to check the former distillation experiment. The solution was boiled at about 90° C., and the distillate collected during the boiling. It appeared as a colorless liquid with an odor similar to that previously described for distillates. It was slightly alkaline, testing P_H 7.5. As indicated in the table, it possessed toxic properties, although the wilting of the plants was not as severe or as rapid as in the residue. Another portion of the residue which was left standing for several days in an open dish, the sides of which were rubbed occasionally with a glass rod, failed to show any crystals. As the distillate gave an alkaline reaction, it seemed worth while to test for organic bases. But tests for amines, previously cited, were negative. As Lathrop (*17*) had reported the finding of aldehyde in a filtrate of *Fusarium cubense* growing upon an Uschinsky solution, tests for aldehydes were made, including the resorcinal test for aliphatic aldehydes, the acenaphthene test for cyclic aldehydes, and the silver-mirror test. All were negative.

The chemical investigation of staling in *Fusarium* sp. conducted by Pratt (*24*) is of considerable interest. When fungi were grown upon Richards' solution, she found that they produced small quantities of alcohol, salts, fatty acids, ammonia, and possibly traces of aldehydes. Staling was not considered to be due to an enzyme, but rather to the simple organic acids which are toxic at low concentrations. In spite of the failure of the writer to obtain calcium salts of organic acids, the subject is worthy of further investigation. Unfortunately, the rush of other work has for the present made impossible any further efforts in this direction.

The positive tests for nitrites in filtrates of 2 or 3 weeks old cultures of *Fusarium vasinfectum* growing upon Richards' solution made it desirable to find out more about this chemical. It is known that nitrites are quite toxic to plants at fairly low concentrations. Was the concentration in the filtrates great enough to be poisonous? Using the official method for the quantitative determination of nitrites, it was found that with three different strains tested at different times the amount of nitrite-nitrogen varied from 0.0125 mgm. to 0.04 mgm. per cubic centimeter. A solution of chemically pure sodium nitrite was made up so as to contain 0.04 mgm. of nitrogen per cubic centimeter. Six cotton seedlings with roots cut under water were placed in this solution, and within 22 hours a marked withering and browning of the stems was noticed, starting at the base and running up for some distance. But when the experiment was duplicated and plants were placed in the solution which had been

made up about 48 hours previously, there was no ill effect to be noted. Nitrites in solution are not very stable, and this probably accounts for the seeming discrepancy. That nitrites in dilute form can produce a wilting and killing of cotton plants was determined in the following manner: One pot of cotton seedlings growing upon a sandy loam soil received 1,000 c. c. of a sodium nitrite solution containing 0.5 mgm. of nitrite-nitrogen per cubic centimeter. The solution was applied in three applications at intervals of 24 hours. Another pot was treated in a similar manner, using potassium nitrite as the salt. A third pot, treated with water, served as a control. Within 48 hours all of the plants, about 40 in number, of the two pots treated with nitrites (see fig. 2) showed droopy cotyledons and

FIG. 2.—Effect of nitrite on cotton plants. Control pot at the right and pot to which 1,000 c. c. of sodium nitrite, 0.5 mgm. of nitrite-nitrogen in each cubic centimeter, at the left. Plants all of the same age and handled similarly. Photographed 48 hours after initial treatment. Twenty-four hours later all the plants in the nitrite pot were dead

leaves, while those of the control pot remained normal. Within three days after the applications, almost all of the plants in the nitrite pots were dead. When one considers the instability of nitrites under soil conditions, the greater concentration applied to soil as compared to those found in the filtrates does not seem objectionable.

The experiment clearly indicates that in the presence of nitrates the fungus produces nitrites which may in part cause the pathological effects. As the fungus is a vascular parasite, it must undoubtedly at times come in contact with nitrates taken up by the roots. Furthermore, as the same fungus is a soil inhabitant, it is quite possible to conceive of nitrite poisoning readily occurring in the absence of any fungal invasion.

OTHER EVIDENCE INDICATING TOXIC ACTION

Aside from evidence of toxic action deduced from a study of filtrates, there is other evidence indicating that wilting is not due to a mere mechanical plugging of the vascular elements by the fungus. It has already been noted that Ajrekar and Bal (*1*) found that by far the greatest number of vessels in a wilted cotton plant are not occupied by the fungus. The writer likewise has spent considerable time in making microscopic observations of wilted cotton plants and can confirm Ajrekar's and Bal's findings. In addition to this, the writer has not infrequently been unable to culture the fungus from wilted plants which possessed discolored vascular elements, even when stem bases and pieces of taproots were utilized. That other investigators have been unable at times to culture *Fusarium vasinfectum*

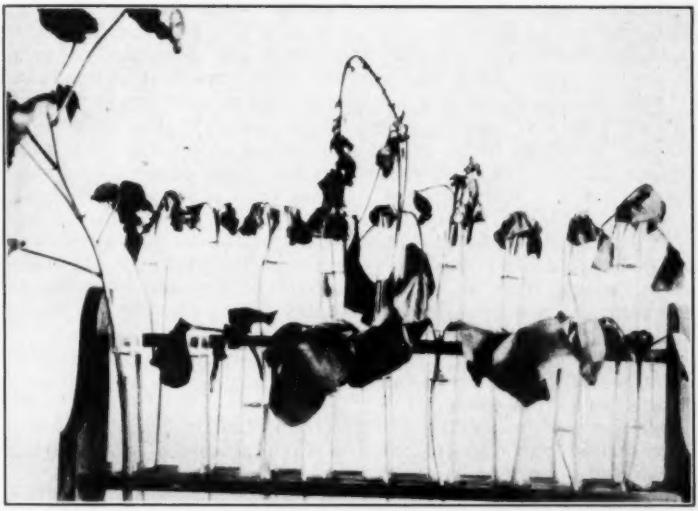

FIG. 3.—Front row: Nine plants with roots removed, after standing 24 hours in uninoculated Richards' solution. Back row: Nine plants with roots removed, after standing 24 hours in a filtrate of Richards' solution in which *Fusarium vasinfectum* had been grown for 3 weeks

from wilted cotton plants is, in view of these observations, not surprising, and Dastur's (*6*) conclusion that wilt is not caused by that fungus was due partly to his inability to culture the fungus from some wilted plants.

CONCLUSION

A study of filtrates with Richards' solution as the medium for the growth of *Fusarium vasinfectum* shows that there are at least two toxic substances present in the filtrate. (See fig. 3.) One is a volatile compound, slightly alkaline in reaction, and the other an inorganic salt, in the form of nitrite. There may be other toxic substances, such as organic acids or alkaloids, but these have not been detected. The finding of nitrites in sufficient quantity to cause poisoning of

cotton plants opens up a field of investigation which has largely been neglected in work with wilt-producing fungi.

As the fungus is not present in sufficient quantity within the xylem to account for the wilting, and as wilting is at times obtainable in the absence of the fungus, it seems logical to conclude that the pathological phenomena here discussed are due primarily to certain poisonous chemical compounds produced by the fungus.

SUMMARY

A large number of experiments are recorded in which pure cultures of different strains of the cotton-wilt fungus were grown on various liquid media, the fungus removed from these, and toxicity studies undertaken with the filtrates.

It is shown that filtrates representing cultures growing on Richards' solution are quite toxic to cotton plants, while filtrates of cultures growing on media containing organic nitrogen, such as Uschinsky's asparagine solution or peptone-beef broth are nontoxic. May not this explain why cotton wilt is much more prevalent in light sandy soils devoid of appreciable quantities of organic nitrogen as compared to richer types of soils? Attention is called to the difference in metabolic products to be expected in media of diverse composition.

When cultures are filtered through fine filter paper the filtrate is found to contain viable spores. It has been determined that while the sterile Richards' solution is markedly acid, testing P_H 4.0 to P_H 4.4, the growth of the cotton-wilt fungus for two to three weeks renders the solution considerably less acid, the hydrogen-ion concentration ranging from P_H 6.0 to P_H 6.6. It appears, however, that this change in acidity is not the factor which renders the filtrates toxic.

When whole plants with root systems carefully handled are placed in filtrates of *Fusarium vasinfectum*, wilting ensues as with plants that are freed from roots.

That the toxic properties of filtrates of Richards' solution are not due to increases in osmotic pressure was determined by diluting the filtrates and observing its action on cotton plants.

Substituting glucose for sucrose in Richards' solution, it is found that the fungus makes as good a growth as in the sucrose medium and that the glucose medium also possesses toxic properties. It was found that uninoculated glucose Richards' solution caused a slight withering of the leaves.

In very bright sunlight, it is recorded that filtrates cause wilting much more rapidly than in diffused light.

By subjecting filtrates to distillation in vacuo as well as to ordinary boiling and testing, the distillates as well as the residues, were found to possess toxic properties, the residue being considerably more toxic. The distillates possess a pungent odor and give an alkaline reaction. Tests for amines and alkaloids in the distillates were negative.

The filtrates of 2 or 3 weeks old cultures on Richards' solution gave positive tests for nitrites. These were found in quantities ranging from 0.0125 mgm. to 0. 04 mgm. of nitrogen for each cubic centimeter of solution. Using chemically pure sodium nitrite solution

comparable to the concentration found in the filtrates, it was determined that this is markedly toxic to cotton plants.

Comparing the action of filtrates of three different species of Fusarium on cotton plants, it was found that a filtrate of *F. tracheiphilum*, the cowpea-wilt fungus, is comparable in toxicity to filtrates of *F. vasinfectum*, while a filtrate of *F. lycopersici*, the tomato-wilt fungus, is considerably less toxic. Attention is called to the close relationship existing between the two first-mentioned species.

Attempts to obtain calcium salts of any organic acid present in the filtrate of the cotton-wilt fungus resulted in failure.

Microscopic observations of the vascular elements of wilted cotton plants clearly indicate that wilting is not due to a mechanical plugging up of the vessels by the fungus.

It is recorded that wilted cotton plants are occasionally found in fields badly infested with the wilt-inducing fungus from which the fungus is apparently absent. It is suggested that the wilting and interior discoloration of the xylem in these cases is due to the formation of toxic substances by the fungus in the soil.

The conclusion is drawn that filtrates of *Fusarium vasinfectum* growing on Richards' solution possess at least two substances poisonous to cotton. One is a volatile compound with an alkaline reaction and the other is an inorganic salt in the form of nitrite.

The wilting of cotton infected with *Fusarium vasinfectum* is considered to be due to poisonous chemical substances formed by the fungus.

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CORRELATED INHERITANCE IN WHEAT[1]

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INTRODUCTION

Since the rediscovery of Mendel's law in 1900, there have been many studies of inheritance in wheat. Of these rather numerous investigations, however, only a few have comprised correlated studies of various characters on the same plant.

Inheritance of awns and of spike density have received some attention, but the studies have hardly more than indicated the problem. Both have been thought by some to be rather simple in their inheritance, and in some crosses such is the case without doubt, but lately there has come a recognition of considerable complexity.

As used in this discussion, the term "density" refers to compactness of the spike. Compact spikes have short rachis internodes, and the spikelets are close together. Such a spike is said to be "dense," whereas one with long rachis internodes, with the spikelets far apart, is said to be "lax."

REVIEW OF LITERATURE

The literature available on the inheritance of awns and spike density in wheat is surprisingly meager. Only four applicable papers on awns and a somewhat greater number on spike density have been found.

AWNS

It has been generally accepted that the awnless condition is dominant over the awned condition in wheat and that the F_1 plants are intermediate in this respect. Several workers report a simple one-factor difference and therefore obtain a 3 : 1 ratio or a 1 : 2 : 1 ratio

Howard and Howard (7)[3] in India found that some wheats behaved in a considerably more complex manner. A fully bearded wheat was crossed with one described by them as being really awnless, a fact which is emphasized as important, inasmuch as the so-called awnless varieties really have short tip awns. In the F_2, five awn classes were obtained: (1) Entirely awnless, (2) short tips, (3) long tips, (4) nearly fully bearded, and (5) fully bearded. When the awned and partially awned plants were grouped together, there was a ratio of 15 awned to 1 entirely awnless. These results were explained on a two-factor basis. Four classes bred true, and when the short-

[1] Received for publication July 29, 1926; issued January, 1927. This paper was submitted to the faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his appreciation for the helpful suggestions of H. K. Hayes concerning the plan of the experiment and the methods of studying the data. Thanks are due to E. C. Stakman and M. N. Levine for cultures of physiologic forms of black stem rust of wheat and for growing two hybrids in the international uniform rust nursery; to O. S. Aamodt for assisting in various ways with the rust investigations; and to D. C. Tingey for growing the various generations of hybrids and for assistance in recording observations and in making calculations.

[3] Reference is made by number (italic) to "Literature cited," p. 1191.

tipped plants and the long-tipped plants were crossed, F_2 segregation showed some fully bearded and some awnless plants as well as the intermediate forms.

Clark (*4*) likewise found a somewhat complex condition in the inheritance of awns of a cross between Hard Federation and Kota. He made five classes of awn types in F_2 and arrived at the conclusion that segregation was too complex to analyze by the methods which he used. He reached the conclusion also that awns followed to a certain extent the maternal parent. This was somewhat irregular, but he felt that his results justified his conclusion.

Recently Nilsson-Leissner (*12*) and Meyer (*9*) have shown in spelt × vulgare crosses that awns are linked with the spelt and with the speltoid forms.

Nilsson-Ehle (*11*) obtained by mutation true-breeding forms of awnless, of half-awned, and of awned wheats. Awnless forms were partly dominant to half-awned and to awned, and half-awned forms to awned. He explained his results on the basis of multiple allelomorphs for half-awned and fully awned plants arising by complex mutation from the awnless plants.

SPIKE DENSITY

In crosses between species of wheat, rather dense spikes have been obtained in unusual ratios, but usually when vulgare wheat has been studied in a cross with other vulgare wheats or with true *Triticum compactum*, rather simple segregations have been found. There are, however, a few complex cases.

Spillman (*14*), Gaines (*5*), Biffen (*1*), and others report simple segregations into 3:1 or 1:2:1 ratios.

Hayes and Harlan (*6*) report that barley internode length is a stable character and segregates in a distinctly reliable manner, a variety of homozygous lines being established in F_3. From crosses between two sorts varying in spike density they obtained true-breeding lines, similar to both parents, in one case without any homozygous intermediate forms and in another case with true-breeding intermediates. Their genetic analysis seems to indicate one main-factor difference in one cross, two factor or three factor differences in others, and in some cases in addition to the main-factor differences some minor modifying factors.

Boshnakian (*2*) found spike density correlated with length of straw. He concluded that compactness in spikes was correlated with a tendency to shorten the plant throughout. Squareheadedness was defined as a condition wherein the number of rachis internodes in the upper third of the rachis bore a ratio of not less than 1.33 to the number in the middle third. Squareheadedness was somewhat affected by environment but was found to be inherited.

Parker (*13*) crossed a *compactum* wheat with a squareheaded vulgare form of intermediate density and obtained forms decidedly more lax than either parent. There was a series of forms of increasing density until the density of the compact parent was reached. Parker thought his paper "clearly pointed out the fact that the problem of the inheritance of the character of laxness and denseness in the ears of wheat is a much more complicated one than was previously imagined." The importance of determining density by measuring internode length instead of making "eye classification" is emphasized.

Nilsson-Ehle (*10*) obtained dense, mid-dense, and lax forms from a cross between compact and mid-dense sorts. The mid-dense parent (squarehead) was recovered in about 1 of every 64 F_2 plants. He explained his results by designating the compact parent as CC L_1L_1 L_2L_2 and the mid-dense parent as cc l_1l_1 l_2l_2. L_1 and L_2 were lengthening factors and C was a factor which produced short rachis internodes and also inhibited the expression of L_1 and L_2.

More recently Nilsson-Ehle (*11*) has reported a mutation of speltoid types in a progeny of a cross of spelt × vulgare. One of his speltoid types again mutated to produce a subcompactum (*11*). Nilsson-Leissner (*12*) also reports similar findings as well as several chimeras in spike density and in branched spikes (ramification).

Meyer (*9*), who measured the length of rachis internodes in several crosses, found in one of them a 1:14:1 ratio of pure compact, intermediate and heterozygous combined, and pure lax. White Frankensteiner (internode length of 5.3 mm.) was crossed with Eckendorfer (internode length, 3.75 mm.). The F_2 plants segregated in forms varying in spike density from 3.74 mm. to 4.94 mm., with the greater number of plants showing intermediate density.

EXPERIMENTAL DATA

The research work herein reported falls naturally into two parts, as follows:

(1) General plant-breeding work on a cross between Dicklow and Sevier varieties of wheat. In this cross detailed genetic data were not obtained, but some yield data were obtained and some strains which were thought to be resistant to *Puccinia graminis tritici* E. & H.

(2) Detailed study of single and correlated characters in a cross between Sevier and Federation varieties of wheat.

DESCRIPTION OF PARENTS

The parents used were Dicklow, a spring wheat commonly grown under irrigation; Federation, a new variety introduced rather recently from Australia; and Sevier, a variety discovered about as recently in Sevier County, Utah.

DICKLOW

Dicklow wheat possesses the spring habit of growth and is medium late in maturing. The stems and leaves display a rather grayish-blue color just before maturity owing to a distinct glaucousness. The stems are rather strong and coarse, and the leaves are broad. The spikes are awnless, though short beards or beaks are rather common at the apex. The glumes are glabrous and white. The kernels are soft, white, and from short to mid-long. The spikes vary from an erect to a nodding position. The shape of the spike most common to the variety is mid-dense and clavate (clubbed at the apex). This latter character varies, depending on environment.

Federation

The variety Federation has great commercial importance in Idaho on irrigated land and is now being brought into Utah, where it is replacing the predominant variety Dicklow on land where lodging is bad. Federation has better standing ability and was therefore chosen as a parent to cross with Sevier, whose standing ability is markedly weak. Federation has a distinct spring habit, matures somewhat earlier than Dicklow, is about 15 to 20 cm. shorter of culm than Sevier and about 6 to 8 cm. shorter than Dicklow.

Its spikes are awnless except for very short tip awns and an occasional apical awn, and the variety is classified by Clark, Martin, and Ball (*3*) as awnless. The spike is somewhat oblong, a trifle more dense than ordinary lax wheats such as Marquis, and is borne erect. The glumes are dark bronze to brown and the kernels are white, soft, and short.

Sevier

The variety Sevier has commercial importance in the Sevier River valley of Utah where there is some black stem rust occasionally. The straw is extremely weak and the grain lodges badly, although unless lodging is extremely severe, there is little loss, for Sevier seems to have a certain amount of resistance to the physiologic forms of *Puccinia graminis tritici* that occur in this region. It is extremely high yielding under favorable conditions, is somewhat drought resistant, and is also thought to be slightly resistant to alkali, though this has not been proved. The spike is awned and is somewhat laterally compressed, and though it is somewhat more dense than the spike of Federation, Sevier can not be classed as a club wheat. The glumes are bronze but not so dark as those of Federation. The kernels are white and in some pure lines are almost as hard as those of durum wheats, whereas in others the kernels are soft. In the pure lines used in the hybrids studied the kernels are hard but not so hard as in some of the other lines. The grain is held firmly in the chaff and no amount of weathering seems to cause shattering, a fact which makes it a desirable parent to use both with Federation and Dicklow, as these two varieties both lose considerable grain if left standing in the field any length of time after maturity.

Dicklow×Sevier cross

In 1919, in an effort to obtain improved strains of wheat, a large number of variety crosses were made between Sevier and Dicklow, the foremost spring wheat grown under irrigation in this region. Both varieties were composite, that is, each contained several or many pure lines, some of which were distinctly visible. In this first cross no record was made as to which plants were used as parents. F_3 and F_4 homozygous lines were obtained, some of which were more dense and others more lax than either parent. Pure-line selections were made in both varieties, and numbers were assigned to each. In 1921 and subsequently, yield data were obtained on these pure lines and on hybrid strains as soon as they became available.

In 1924 nursery tests were made under irrigation and in 1925, under dry farming. Table 1 gives the yield data for the irrigation test and Table 2 those for the dry-farm test.

In both tables it is apparent that high yields and good straw strength were obtained in certain of the hybrids. In both cases, also, some of the best-yielding lines have weak straw. In the dry-farm tests, Sevier yields were very high, but this wheat is excessively weak-strawed. There is considerable economic promise in the hybrids both for irrigated farming and for dry-farming. For example, IV C 15, III C 8, and F 68 were thought to be promising and are now being tested in plats. In the tests with irrigated spring wheat, III C 18, F 22, F 68, and V C 8 have been used in plot tests. G 149 has proved very rust resistant.

TABLE 1.—*Yields of superior strains of Dicklow×Sevier hybrid wheats, odds that they will be better yielders than Dicklow, shape of spike, awn condition, and straw strength (perfect erectness=100 per cent)*

[Irrigated spring wheat, 1924; average of four 3-row blocks, at Logan, Utah]

Spike shape	Pedigree	Awns	Acre yield	Per cent erectness	Odds
			Bushels		
Compact club	III C 18	[a] A	70.1±3.35	83	1500:1
Do	G 38	[b] a	68.9±3.29	76	
Medium club	G 205	a	68.5±3.27	62	
Faintly compact	F 68	A	68.5±3.27	83	
Compact club	P 72	A	67.9±3.25	71	
Do	F 22	A	67.1±3.21	92	
Medium club	G 148	[a] A	67.0±3.20	75	400:1
Long loose	G 218	A	67.0±3.20	70	
Compact club	IV C 2	A	66.8±3.19	85	
Medium club	G 97	a	66.7±3.19	78	
Faintly compact	C 18	A	65.9±3.15	57	
Medium club	C 16	A	65.7±3.14	75	
Compact club	V C 8	A	65.7±3.14	80	
Do	D 10	a	65.8±3.12	74	
Long loose	G 175	a	65.0±3.11	82	
Do	G 209	a	65.0±3.11	56	145:1
Medium club	F 32	A	64.7±3.09	80	142:1
Compact club	F 24	A	64.6±3.09	89	
Long loose	G 149	A	64.5±3.08	61	
Do	A 4	A	64.5±3.08	78	
Do	A 4	A	64.5±3.08	78	
Do	G 112	a	64.3±3.07	47	
Do	P 42	a	64.0±3.06	77	
Do	G 27	A	63.8±3.04	80	
Medium club	D 9	Aa	63.5±3.04	76	
Long loose	G 49	A	63.1±3.02	73	
Do	C 10	a	63.1±3.02	82	100:1
Do	G 31	A	63.0±3.01	77	
Do	C 22	a	63.0±3.01	65	
Compact club	P 14	Aa	62.3±2.97	80	
Long loose	G 212	A	62.0±2.96	48	
Do	G 121	a	61.8±2.95	43	
Compact club	G 180	A	61.7±2.95	75	
Do	G 84	A	61.7±2.95	80	
Medium club	G 11	Aa	61.6±2.94	80	
Do	C 14	a	61.5±2.94	78	
Do	C 9	a	61.2±2.93	82	
Faintly compact	D 5	A	61.2±2.93	61	
Compact club	G 122	a	61.0±2.92	80	
Do	V C 1	A	61.0±2.92	79	30:1
Do	G 126	A	61.0±2.92	75	
Do	Dicklow	a	49.2±2.35	79.6	(Av. 50)

[a] A=awns [b] a=awnless. [c] Aa=heterozygous for awns.

TABLE 2.—*Acre yields and percentage erectness of pure lines of Sevier, of hybrid strains of Dicklow×Sevier, and of the commonly grown dry-farm varieties*

[Dry-farm winter wheat; average of three 3-row blocks grown in 1925 at Logan, Utah]

Strain	Acre yield	Per cent erectness	Strain	Acre yield	Per cent erectness
	Bushels			*Bushels*	
Sevier 86	60.1±3.08	40	Sevier 34	48.6±2.49	40
V C 8	58.7±3.01	58	F 22	47.8±2.45	83
IV C 15	57.7±2.96	85	Kofod	47.6±2.44	7
V C 2	57.0±2.92	33	S 33	47.6±2.44	88
Sevier A	56.9±2.92	25	P 14	47.4±2.43	88
P 72	56.8±2.91	62	Sevier 59	47.3±2.43	23
Sevier 161	55.7±2.86	23	Sevier 56	47.0±2.41	38
III C 8	55.4±2.84	83	S 26	46.7±2.40	85
S 27	55.3±2.84	75	G 40	46.4±2.38	45
G 38	54.7±2.81	77	B 5	45.9±2.35	20
Sevier 125	54.5±2.80	20	S 39	45.8±2.35	92
F 68	54.1±2.79	80	Sevier 31	45.8±2.35	12
Sevier 40	53.9±2.76	25	Turkey	45.7±2.34	38
III C 18	53.4±2.74	63	P 6	45.3±2.32	85
Kanred	53.4±2.74	20	G 122	44.2±2.27	83
Sevier 47	52.9±2.71	38	Odessa	43.4±2.23	25
Sevier 128	52.5±2.69	33	G 175	42.7±2.19	70
IV C 9	51.6±2.65	92	R 128	42.3±2.17	80
Sevier 83	51.4±2.64	8	S 21	41.9±2.15	78
IV C 2	51.3±2.63	87	Silver Coin	40.6±2.08	87
G 84	51.3±2.63	60	R 152	40.1±2.06	83
F 32	51.2±2.63	58	G 148	38.9±2.00	83
P 144	51.1±2.62	90	Gold Coin	38.1±1.96	92
P 42	51.1±2.62	75	F 42	37.3±1.91	68
G 149	48.8±2.50	50	G 97	35.6±1.83	88
F 24	48.8±2.50	77	Jones Winter Fife	35.3±1.81	78

RUST TESTS

In 1924, 55 pure lines from the cross Dicklow×Sevier were grown in the rust nursery at University Farm, St. Paul, Minn. Of these, two were found to be highly resistant. Three or four were segregating for resistance, and all the others were highly susceptible. The two resistant strains were G 40 and G 149.

During the spring of 1925 seedlings of those two strains were subjected to greenhouse rust tests in which all the physiologic forms of black stem rust of wheat then available in the pathology greenhouse at University Farm were used. Altogether 19 forms of rust were employed. Under each variety in Table 3, where the data are tabulated, the types of seedling infection are listed. Type 4 is a large uredinial pustule with little or no chlorosis. It indicates complete susceptibility. Type 3 is a smaller pustule with some chlorosis. Type 2 is a small pustule inclosed in a considerable area of hypersensitive tissue. Type 1 is an extremely small pustule with a distinct margin of necrotic tissue. The most resistant type is not a pustule at all, but only sharp flecks, indicating that the rust has infected the leaf but has been overcome and thrown off. Such infection is designated "zero flecks" (0 f).

TABLE 3.—*Relative resistance of two Dicklow × Sevier hybrids, as compared with that of their parents and of Marquis, to various physiologic forms of Puccinia graminis tritici*

[Tests at University Farm, St. Paul, Minn., 1925]

Physiologic form	Date observed	Marquis seedlings showing infection of type—					Dicklow seedlings showing infection of type—					Sevier seedlings showing infection of type—					G40 seedlings showing infection of type—					G149 seedlings showing infection of type—				
		0	f	1	2	3	4	0	f	1	2	3	4	0	f	1	2	3	4	0	f	1	2	3	4	0	f	1	2	3	4
1	May 15						12						11					12					15						14		
	June 20						10																							8	
3	May 16												10																		
	June 18												11																	11	
	do																												2	10	1
9	May 12					8							11					12					4	5			1	7	3	
	June 20												10																13		
10	May 19												12														2		7		
	June 19												11																	10	
	do												12																	10	
11	May 14											14				4	8					2	13			9	4				
	June 17												14																	15	1
15	May 17												9				7	3				4	5	2		10					
	June 20												13																	16	
Pusa 17	May 23												14				6	7					4	4			5		5		
Minn.	June 1						15						16				14				13						16		Photo		
Pusa	June 18												11													10			2		
18	June 11						11					1	10				8	5				2	10					9	3		
	June 19												12																	13	1
	do												13																	14	
19	June 10												11				10	3				2	13				2	10			1
	June 17												13																	11	
21	May 24					5	7					1	11				8	4					9	3				11	3		
	June 17												13																	12	
24	June 22												10																	10	
27	May 30									1	2	7				11	2				2	5	5			2	3	9			
	June 18												13																5	8	
29	May 25						13						14			2	13					1	8	3				11	3		
	June 17												12																	14	
30	May 29						13						15				14						14				13	1			
	June 22												13													2			7		
32	June 10												11				9						9					13			
	do																											11			
	June 22												8										10					11			
36	May 8												14				6	5					6	3		1		8	2		
	June 19												12															12	5	1	
	do																											14		1	
38	May 16				12								13				12							12				16			
	June 18												12																	11	
39	May 18				10								14				13					4	11					12	2		
	June 18												10					11											10	1	
40	May 23						11						14										5	5					3	9	
	June 19												9																	13	

Dicklow was found to be completely susceptible to all 19 physiologic forms of *Puccinia graminis tritici*, whereas the Sevier parent showed some resistance. Pure line G 40 showed about the same resistance as did Sevier. Pure line G 149, however, was on the whole considerably more resistant than either parent (see fig. 1). It is worthy of notice that G 149 was resistant to every form with which it was inoculated, which included all of the forms that have been known to cause epidemics in the United States. It is notably resistant to rust forms 9, 17, 32, and 36, the last one of which severely damages Kanred. Because it arose in a varietal cross the exact parentage of G 149 is not known.

Leaf-rust observations were also taken in 1925 at University Farm. About 75 per cent of the Marquis wheat was infected as compared with 80 per cent of the G 40 and 22 per cent of the G 149. Tests by Melchers of 13 strains of the same cross in Kansas in 1924 showed 10 completely susceptible, 1 slightly resistant, 1 moderately resistant, and 1 resistant, i. e., "many sharp flecks, few minute uredinia."

Just occasionally, it seems, there occurs in the progeny of this cross a plant resistant to stem rust and to leaf rust.

In 1925 another set of pure lines from the Dicklow×Sevier cross was grown in the rust nursery at St. Paul. G 40 and G 149 were included and were again found to be highly resistant. One additional pure line, G 84, was found to be resistant, fully as resistant so far as this one test is concerned as G 149. Many other pure lines were segregating for resistance to rust. Resistant plants from the segregating row of G 212 grown in 1924 were sown separately in 1925.

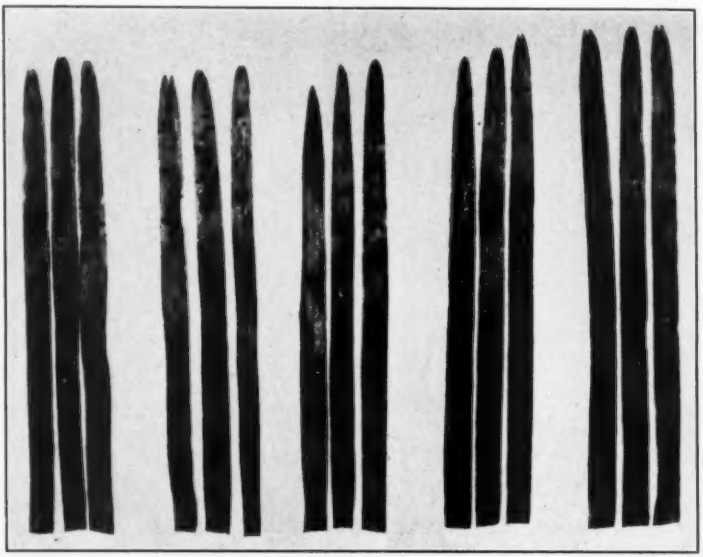

FIG. 1.—Reaction of Marquis, Dicklow, hybrid G 149, hybrid G40, and Sevier wheats to physiologic form 17 of *Puccinia graminis tritici* in the greenhouse. Marquis (extreme left) was used as a control and gave a 4 reaction. Dicklow, one of the parents (second from left), gave a strong 4 reaction (fully susceptible). Sevier, the other parent (extreme right) gave a 3 reaction (somewhat resistant). One of the hybrids between Dicklow×Sevier, G 40 (second from right), gave a 1 reaction. The other hybrid, G 149, gave 1 and "zero-fleck" reactions (highly resistant). G 149 was moderately to highly resistant to all the 19 physiologic forms available for test

All of these were infected to about the same extent as G 40, G 84, and G 149. The artificial epidemic in 1925 was produced by spraying with spores of physiologic forms 1, 3, 9, 15 (?), 17, 18, 19, 21, 29, 30, 36, 38, and 39.

During the summer of 1925 both pure lines G 40 and G 149 were grown in the international uniform rust nurseries of the United States and Canada. A summary[3] of the tests is given in Table 4. Ceres, Kota×Marquis, Marquillo, Kota, and Progress showed marked resistance as compared with Marquis. The percentages of infection in Kota, Kota×Marquis, and Ceres were, respectively, 6.59, 4.92, and 8.62, as compared with 26.60 in Marquis and 30.89 in Preston. In this same test the percentage of rust in G 40 and G 149 was 4.15 and 3.88, respectively, G 149 showing the least rust of any strain of *Triticum vulgare* tested in 1925.

[3] LEVINE, M. N., and STAKMAN, E. C. [REPORT ON STEM RUST INFECTION OF WHEAT AND OATS GROWN AT DIFFERENT STATIONS IN THE UNITED STATES AND CANADA IN 1925.] 4 tables. [1926.] [Mimeographed].

TABLE 4.—*Average percentage of stem rust on 16 varieties and strains of spring wheat grown in uniform rust nurseries at different stations in the United States and Canada in 1925* [a]

Class, variety, and cereal investigation number	Calculated percentage of infection						
	Region						Average for all regions
	Prairie	Great Plains	Great Lakes	Southwest	Southeast	East Canada	
Hard Red:	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Haynes, 2874	54.8	25.2	18.9	5.1	0.1	T. +	23.95
Marquis, 3641	55.8	31.2	24.4	6.0	.3	T. −	26.60
Power, 3697	63.6	30.6	23.9	8.7	.3	T. +	28.71
Ruby, 6047	45.1	28.7	24.3	6.0	.3	2.0	23.32
Marquillo, 6887	18.5	11.7	8.6	0	T. −	0	9.80
Preston, 3081	68.5	35.7	22.1	7.6	.1	T. +	30.89
Kota, 5878	15.0	6.8	4.7	2.6	T. −	T. +	6.59
Progress, 6902	24.2	7.7	6.9	0	0	T. −	10.12
Kota×Marquis, 6898	11.0	3.9	4.4	T. −	0	T. +	4.92
Marquis×Kansas, 7370	52.7	26.7	16.6	0	T. −	T. +	24.93
Ceres, 6900	17.3	9.5	7.0	0	T. −	0	8.62
Parker×Minnesota, 2222	37.2	21.0	3.5	0	----------	B. T.	20.45
Weighted average for Hard-Red group	38.6	19.9	13.7	5.0	.1	.2	18.43
White:							
Quality, 6607	36.5	22.3	18.1	0	.3	.1	19.39
Little Club, 4066	66.9	33.2	27.1	14.2	.1	.2	31.21
Dicklow×Sevier, G 40	9.7	1.8	1.0	0	----------	0	4.15
Dicklow×Sevier, G 149	9.7	1.2	.5	0	----------	0	3.88
Weighted average for White group	30.7	14.6	12.5	8.9	.2	1	16.06

[a] From mimeographed report of Levine and Stakman for the crop season of 1925. LEVINE, M. N., and STAKMAN, E. C. Op. cit.

GENETIC STUDY

Inasmuch as the Dicklow×Sevier cross proved extremely promising from the standpoint of wheat improvement, a cross between a pure line of Federation and a pure line of Sevier (Sevier 60) was used for a genetic study of spike density and awns. Observations were also made on the length and number of culms, on squareheadedness, and (in F_2) on thickness of neck, i. e., the diameter of culm measured just below the spike.

The cross was made in 1922 at the Utah Agricultural Experiment Station, Logan Utah. The F_1 plants were grown at Logan in 1923 and the F_2 plants at Logan in 1924. The writer entered the University of Minnesota in June, 1924, and after harvest, shipped the plants to University Farm, St. Paul, the plants having been pulled by the roots and packed carefully.

The hybrid plants were in 8 families, 5 of one cross (Sevier 60 × Federation) being numbered consecutively from 1 to 5. Family 4 was the largest and most uniformly developed family, and for this reason was chosen for the most detailed study. Observations were taken on families 1 to 3 in a somewhat, but not exactly, similar manner. Families 6, 7, and 8 were of another cross, Sevier G 101 ×Federation.

METHOD OF TAKING OBSERVATIONS AND STUDYING DATA

The lengths of the culms were determined by placing the roots against a footboard nailed perpendicular to another board laid flat on a table. Centimeters were marked on the table board. This permitted a rapid reading of the length of the culm to the base of the spike. The length of the longest culm was read first and that of each succeeding shorter one in order.

The plants were classified with respect to awn types into groups 1, 2, 3, and 4. The plants in group 1 had only short beak awns, with an occasional apical awn. The intention was that this class should be comparable with Federation. Group 2 consisted of plants with intermediate awns, as nearly as possible like those of the F_1 plants. Group 4 was fully awned, as is Sevier, whereas group 3 was part way between groups 2 and 4. In group 3 the awns varied considerably in length, the apical awns were full length in some cases, but toward the base the awns were very short. There was so much variation in length of awn, especially in groups 2 and 3, that definite separation was difficult. Spikes showing the range of each awn class were kept constantly at hand to assist in classifying any given plant.

The length of the rachis in millimeters was taken from the node at the base of the spike to the base of the uppermost spikelet. The number of spikelets attached to the upper third and the number attached to the middle third of the rachis were counted. The ratio between these became the index of squareheadedness. Thickness of neck was measured on a leading culm with vernier calipers to the nearest tenth of a millimeter. Spike density was ascertained by measuring in millimeters on the spike of the same leading culm the length of that part of the rachis that extended from the base of one spikelet to the base of the sixth one above, i. e., the length of 10 rachis internodes. Care was taken to avoid the internodes near the base and those near the summit of the spike lest these should vary in length from those near the middle.

For the F_3 generation, which was grown at Logan, Utah, in 1925, random selections were made from plants which were represented on certain sections of the spike-density curve of family 4. Twelve sections on the curve (see fig. 3, top) were selected for study, and the first six plants, as they occurred in the record book, were taken as a random sample of that section of the curve. These were sown in the order in which they occurred in the record, beginning with the plants represented by the more dense end of the curve.

The F_3 generation lines of family 4 were sown in rows 1 foot apart and 12 feet long, about 50 kernels to the row, which resulted in about 40 mature plants. Each parent was sown in a similar manner in every fifth and sixth row. In families 5 and 6 all the plants were sown, 10 progeny rows being followed by 1 row of each parent.

In family 4 the same data were collected in F_3 as in F_2, save that the thickness of neck was not measured. In family 5 only the awn data and the length of 10 rachis internodes were obtained, and in family 6 only the awn data.

The F_2 data for each plant were listed on a separate card, and each character was studied by itself. Afterward, a correlated study of the most important characters was made by calculating the coefficient of correlation (r) and the correlation ratio (η).

Awns

In the cross between a pure line of Federation × Sevier 60, F_2 families 1 to 5, inclusive, were classified for the four awn types previously described. Figure 2 shows the range in awn length for each of the four awn classes. The number of plants in each awn group in the five F_2 families is shown in Table 5.

FIG. 2.—The four classes of awns in the Federation×Sevier cross. On the extreme right are the two parents, Federation (above) and Sevier (below). (*Above, left to right*). Awn class 1 (awnless); awn class 2 (short awn tips); awn class 3 (short-tip awns in lower half of spike and part-length awns in upper half); awn class 4 (fully awned). (*Below, left to right*). Awn class 4; awn class 3; awn class 2, and awn class 1

TABLE 5.—*Classification of five families of F_2 plants according to four awn classes. (Sevier 60×Federation)*[a]

Family	Number of plants in awn classes			
	1 Awnless	2 Short awn tips	3 Awns short at base of spike but longer at apex	4 Fully awned
1	46	194	52	56
2	38	90	21	23
3	41	63	24	29
4	91	192	41	69
5	74	106	18	50

[a] See fig. 2 for illustration of awn classes.

In F_3 there were 74 separate progenies of F_2 plants chosen at random from 12 selected sections of the spike-density curve of family 4. (See fig. 3.) Separate F_3 progenies of all the F_2 plants in families 5 and 6 were grown. There were 248 F_3 families in family 6 and 245 in family 5.

In family 4, the 74 F_3 families consisted of progenies of 14 F_2 plants classified as having awns 1, 37 plants classified as having awns 2, 7 as awns 3, and 16 as awns 4. In order to show the nature of awn behavior in the F_3 families, the detailed awn data for family 4 are given in Table 6.

TABLE 6.—*F_3 breeding behavior of plants classified in F_2 into 4 classes on the basis of awn characters*

[Family 4; grown in 1925 at Logan, Utah]

Classified when planted as being in awn class	Number of plants in awn classes—				Classified when planted as being in awn class	Number of plants in awn classes—			
	1	2	3	4		1	2	3	4
1	35	5			2	1	28		11
1	13	17	3	6	2	25	8	7	
1		40			2	4	16	12	8
1	35	4	1		2	11	17	6	8
1	24	14	2		2	3	25	6	6
1	20	19	1		2	8	9	4	7
1	7	14	19		2		12	14	14
1	13	16	10	1	2	9	19	4	7
1	28	11			2	1	20	10	9
1	23	4	12		2	25	11		
1	33				2			40	
1	19	21			2	6	11	16	7
1	26				2	34	2	4	
1	27				2		21	8	11
2	17	7	5	11	3	14	15	11	
2	1	28	1	10	3		7	33	
2		18	4	3	3	14	18	7	
2	1	20	6	11	3		39		
2	27	7		6	3	9	21	10	
2	21	6	3	9	3	35	4	1	
2	27	5	8		3	4	6		
2	16	6	4	10	4				36
2	3	28		9	4			1	36
2	11	2	6		4				40
2	30	4	6		4				40
2	17	8	5	10	4				40
2	11	15	6	8	4		2	7	31
2	29	6	5		4				31
2		40			4				34
2	11	7	7	10	4			5	35
2	40				4		1	3	36
2	11	8	15	6	4		6	9	25
2	4	11	14	11	4			2	38
2	1	33	6		4		5	13	22
2	18	12	9	1	4			9	31
2	10	12	8	10	4			21	19
2	11	17	8	4	4				34

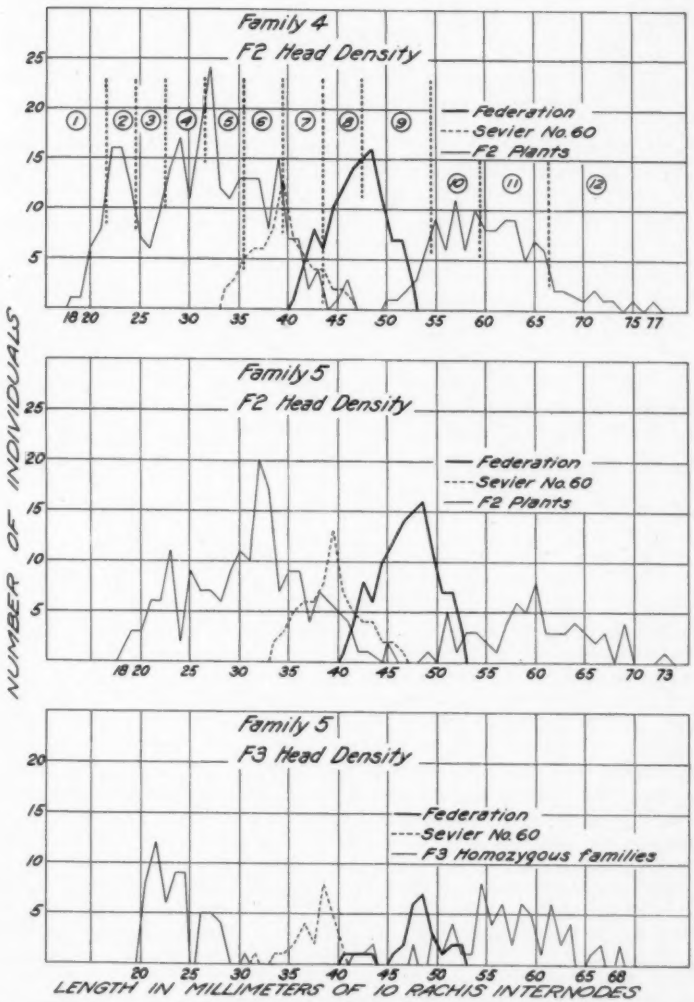

FIG. 3.—Spike density curves of the Federation and the Sevier parents and of F_2 and F_3 families. (Top) F_2 spike density curve of family 4, with curves of parental spike density superimposed. Vertical dotted lines divide the F_2 curve into 12 sections; 6 or 7 of the plants which each section represents were chosen at random to be used as mother plants of F_3 progenies. (Middle) F_2 spike density curve of family 5, with the curves of parental spike density superimposed. Grain of all the F_2 plants of family 5 was sown for F_3 progenies. (Bottom) Curve of mean spike density of homozygous F_3 families, with the curves of mean density of parental rows superimposed. All heterozygous families are omitted

The F_3 showed the following behavior for awn classes:

Number of F_3 families breeding true for—		Number of F_3 families segregating for—	
Awns 1	4	Awns 1, 2	4
Awns 2	2	Awns 1, 2, 3	12
Awns 3	2	Awns 1, 2, 3, 4	25
Awns 4	7	Awns 2, 3, 4 (or 2, 4)	12
		Awns 3, 4	6

In family 5, the seed of 247 F_2 plants was sown, one being omitted because of too few kernels. The plants of each F_3 family were classified according to awns. A summary of the data is given below:

Number of F_3 families breeding true for—		Number of F_3 families segregating for—	
Awns 1	18	Awns 1, 2	41
Awns 2	7	Awns 1, 2, 3	17
Awns 3	4	Awns 1, 2, 3, 4	66
Awns 4	18	Awns 2, 3, 4 (or 2, 4)	49
		Awns 3, 4	7

In family 6, an F_3 progeny of every F_2 plant was likewise grown, each in a single row. The 40 or so plants in each row were classified and the data summarized by families, as follows:

Number of F_3 families breeding true for—		Number of F_3 families segregating for—	
Awns 1	29	Awns 1, 2	57
Awns 2	7	Awns 1, 2, 3	4
Awns 3	0	Awns 1, 2, 3, 4	70
Awns 4	21	Awns 2, 3, 4 (or 2, 4)	46
		Awns 3, 4	11

An examination of the summaries of awn behavior shows that in the F_2 generation of families 4 and 5 there occurred 9 genotypes of plants according to awn-breeding behavior in F_3. Four of these bred true and 5 segregated each in a distinct manner. In family 6 there were 8 genotypes, no F_3 family being found that bred true to awns 3.

On a basis of total F_3 families, the ratios of families that bred true for awns in various ways suggest a two-factor difference for awns. A strictly independent segregation would give approximately equal numbers in each of the four classes that bred true. There are, however, uniformly greater numbers in awn classes 1 and 4—the parental types—than in 2 and 3. This, and the distribution in the segregating classes, suggested linkage. It was found that as good fits as could be expected were obtained when the two factors for awns were considered to be so linked as to produce gametes in the proportion of 1.8 : 1 : 1 : 1.8.

Let us designate full awns, such as occur in the Sevier parent, by *AA TT* (awn class 4); long apical awns but short lateral awns (awn class 3) by *AA tt*; short but well developed tip awns (awn class 2) by *aa TT*; and awnlessness such as occurs in the Federation parent by *aa tt* (awn class 1). In strictly independent inheritance the four gametes *AT*, *At*, *aT*, and *at* would be produced in equal numbers.

In the degree of linkage here suggested, the gametes are produced 1.8 *AT*: 1.0 *At*: 1.0 *aT*: 1.8 *at*. On this hypothesis the number and sort of gametes produced in F_1 and the number and sort of zygotes which would be expected in F_2 are given in Table 7.

TABLE 7.—*Theoretical number and sort of gametes produced by F_1 plants; theoretical number and sort of zygotic combinations resulting in F_2*

	1.8 *A T*	1.0 *A t*	1.0 *a T*	1.8 *a t*
1.8 *AT*	3.24 *AA TT*	1.8 *AA Tt*	1.8 *Aa TT*	3.24 *Aa Tt*
1.0 *At*	1.8 *AA Tt*	1.0 *AA tt*	1.0 *Aa Tt*	1.8 *Aa tt*
1.0 *aT*	1.8 *Aa TT*	1.0 *Aa Tt*	1.0 *aa TT*	1.8 *aa Tt*
1.8 *at*	3.24 *Aa Tt*	1.8 *Aa tt*	1.8 *aa Tt*	3.24 *aa tt*

(1) 3.24 *AA TT*.
(2) 3.6 *AA Tt*.
(3) 3.6 *Aa TT*.
(4) 8.48 *Aa Tt*.
(5) 1.0 *AA tt*.
(6) 3.6 *Aa tt*.
(7) 3.6 *aa Tt*.
(8) 1.0 *aa TT*.
(9) 3.24 *aa tt*.

The expected breeding nature of the 9 genotypes is indicated here:

(1) Breed true for awns 4.
(2) Segregate for awns 3, 4.
(3) Segregate for awns 2, 3, 4.
(4) Segregate for awns 1, 2, 3, 4.
(5) Breed true for awns 3.
(6) Segregate for awns 1, 2.
(7) Segregate for awns 1, 2.
(8) Breed true for awns 2.
(9) Breed true for awns 1.

Of the 9 sorts of genotypes (1), (5), (8), and (9), are the true-breeding forms for awn classes 4, 3, 2, and 1, respectively. Genotype (4) is of the same composition as the F_1 and its phenotype essentially like awns 2. Genotypes (2) and (3) are each homozygous for one dominant factor and heterozygous for the other, (2) being heterozygous for *Tt* and (3) for *Aa*. The progeny of these two groups of plants apparently have a range in awn length that extends from class 3 to class 4. Since these two types of segregates could not be satisfactorily separated in F_3 they were placed together in one class. Genotypes (6) and (7) differ only in that (6) is homozygous recessive for *tt* and heterozygous for *Aa*, whereas (7) is homozygous recessive for *aa* and heterozygous for *Tt*. These two groups were also deemed nonseparable and were therefore placed in one group.

The F_2 genotypes of families 4, 5, and 6 were determined by the F_3 breeding behavior, and the F_2 genotypes were classified for awns on the basis of F_3 breeding behavior. Families 4 and 5 were from crosses of the same pure lines, but family 6 was from a cross of the same pure line of Federation by a different line of Sevier. Since family 5 consisted in F_3 of progenies of all the F_2 plants, it was studied first. Its closeness of fit to the calculated expectancy on the basis of linkage, with 35 per cent crossing over, is shown in Table 8, where the calculated expectancy, the observed numbers, X^2, and P are given. Similar data and the closeness of fit for families 4 and 6 are given in Tables 9 and 10, respectively.

TABLE 8.—*Calculated (C) and observed (O) numbers of plants in each awn group of F_2 genotypes as determined by the F_3 breeding behavior*

[Family 5; grown in 1925 at Logan, Utah]

Genotype	C	O	C–O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
AA TT	23.28	18	5.28	27.8784	1.1975
AA Tt	51.89	56	−4.11	16.8921	.3255
Aa TT					
Aa Tt	61.12	66	−4.88	23.8184	.3897
AA tt	7.21	4	3.21	10.3041	1.4291
Aa tt	51.89	58	−6.11	37.3321	.7194
aa Tt					
aa TT	7.21	7	0.21	.0441	.0061
aa tt	23.28	18	5.28	27.8784	1.1975
Total	226	227	P=.5118	X^2=5.2656	

TABLE 9.—*Calculated (C) and observed (O) numbers of plants in each awn group of F_2 genotypes as determined by the F_3 breeding behavior*

[Family 4; grown in 1925 at Logan, Utah]

Genotype	C	O	C–O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
AA TT	7.65	7	0.65	0.4225	0.0552
AA Tt	16.99	18	−1.01	1.0201	.0600
Aa TT					
Aa Tt	20.01	25	−4.99	24.9001	1.2444
AA tt	2.36	2	0.36	.1296	.0549
Aa tt	16.99	16	.99	.9801	.0577
aa Tt					
aa TT	2.36	2	.36	.1296	.0549
aa tt	7.65	4	3.65	13.3225	1.7415
Total	74	74	P=0.7734	X^2=3.2686	

TABLE 10.—*Calculated (C) and observed (O) number of plants in each awn group of F_2 genotypes as determined by the F_3 breeding behavior*

[Family 6; grown in 1925 at Logan, Utah]

Genotype	C	O	C–O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
AA TT	25.31	21	4.31	18.5761	0.7339
AA Tt	56.25	57	−0.75	.5625	.0100
Aa TT					
Aa Tt	66.25	70	−3.75	14.0625	.2123
AA tt	7.81	0	7.81	60.9961	7.8100
Aa tt	56.25	61	−4.75	22.5625	.4011
Aa tt					
aa TT	7.81	7	0.81	.6561	.0840
aa tt	25.31	29	−3.69	13.6161	.5380
Total	245	245	P=.1349	X^2=9.7893	

Tables 8 and 9 show that families 4 and 5 are very close fits to the calculated expectation on the hypothesis of two linked factors with a crossing over of 35 per cent. The value of P for family 5 was 0.5118, which shows that in 51 cases out of 100 a worse fit might be expected

from chance alone. Family 4 gave a little higher value for P, indicating another almost perfect fit. In family 6, however, P was only 0.1349, which indicates that, owing to random sampling, a worse result might be expected once in 7 or 8 trials.

It may therefore be safely concluded that the F_2 genotypes as classified by the F_3 breeding behavior rather highly substantiates the presence of two factors for awns, both in the same chromosome, with crossing over to the extent of about 35 per cent.

Three things must be done, however, to establish definitely the correctness of the hypothesis. (1) The linkage must be tested. If the true-breeding forms for awns 2 and 3 were crossed, the linkage could be tested, since the factors would then be linked in the opposite way, i. e., in a repulsion series. The expected gametic ratio would then be 1.0 *AT:* 1.8 *At:* 1.8 *aT:* 1.0 *at.* A larger number of forms breeding true for awn classes 2 and 3 than for awn classes 1 and 4 would be expected. (2) A back-cross between the F_1 and the parental homozygous double recessive must be made. This would show in the next generation the ratio of gametes produced by the F_1 plant. (3) A cross between the truebreeding forms of awns 2 and 3 and the double recessive Federation parent must be made. This would demonstrate the correctness of a one-factor difference between each of these and Federation. In this third test it would also be interesting to know the behavior of the progeny of the same crosses with the double homozygous dominant as well as with the recessive.

Spike Density

All of the 8 F_1 plants produced semiclubbed spikes, i. e., though they were not measured for internode length, they were visibly of intermediate spike density. The F_2 showed a wide and striking variation in average length of rachis internodes, some segregates being far more compact and others far more lax than was the case with either parent.

Table 11 gives the data on length of internode for families 4 and 5 and for a small number of plants of each parent grown in a single row in the same part of the field as the F_2 families.

In F_3, 74 plants of F_2 were selected at random from various parts of the frequency curve for spike density of family 4 (see fig. 3, top). The F_3 planting plan was so arranged that each four hybrid F_3 families had a row of Federation and one of Sevier on each side, i. e., there was a row of each parent then four hybrid rows followed by a row of each parent and these by four hybrid families until all were seeded. There were about 40 plants in each row, a leading spike of each of which was measured for density. The same procedure was followed for family 5, save that progeny of all the F_2 plants were sown and the parents occurred only after each 10 F_3 rows. Two hundred and forty-seven F_3 progenies of family 5 were tested for spike density. (See fig. 3, middle.)

TABLE 11.—*Number of plants of Sevier, of Federation, and of the F_2 hybrids between Sevier and Federation grouped into classes according to the average length of rachis internodes (the length in millimeters of 10 internodes was measured in the middle of the head of one main culm on each plant and the length of one internode obtained by pointing off 1 decimal place)*

[Grown in 1924 at Logan, Utah]

Strain	Number of plants in classes of spike density											
	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75	7.25	7.75
Federation						29	68	17					
Sevier				5	38	22	3						
Family 4		58	54	65	62	20	4	13	42	39	17	7	2
Family 5	4	28	38	65	35	11	3	12	18	21	12	1	

To determine how soil heterogeneity affected the variability of the mean internode lengths of Sevier and Federation, coefficients of correlation were calculated for the adjacent rows of Sevier and Federation 1 foot apart, for the rows of Sevier 6 feet apart, and for rows of Federation 6 feet apart. The same calculations were made for family 5. In family 5, however, the two Sevier rows and the two Federation rows were 12 feet apart. The coefficients of correlation (r) are given in Table 12.

TABLE 12.—*Correlation coefficients of spike density between rows of Sevier and Federation 1 foot apart and between each pair of two rows of Sevier and Federation 6 feet apart, in family 4, and 12 feet apart in family 5*

[Grown in 1925 at Logan, Utah]

Strains	Coefficient of correlation in family 4	Coefficient of correlation in family 5
Federation×Sevier	+0.421	+0.579
Sevier×Sevier	+.208	−.092
Federation×Federation	−.104	−.004

Table 12 shows that the spike density of adjacent rows is definitely correlated, but that rows 6 feet apart and 10 feet apart do not vary significantly in the same direction. The results indicate the extent of variation in spike density which results from the soil heterogeneity of the field. The amount to which total variability in mean internode length is dependent upon soil heterogeneity can be determined by substituting r in the equation $v=1-\sqrt{1-r^2}$, where $v=$ variation in percentage. In these tests the variation is found to be from about 10 to 15 per cent.

The mean spike density of each F_3 family was obtained, and the coefficients of variability (C. V.) of the individual rows were calculated. A study of this constant for each family showed whether the family was breeding essentially true or was segregating. Table 13 shows the mean spike density classes and the coefficient of variability classes of rows of each parent and of three groups of F_3 hybrids, namely, those breeding essentially true for dense spikes; those

segregating for spike density; and those breeding essentially true for lax spikes. Table 14 gives similar data for family 5.

The parent rows sown with the hybrids of family 4 had coefficients of variability (C. V.) ranging from 4.50 per cent to 9.34 per cent in the case of Federation, with a mean coefficient of variability of 7.40 per cent for the 24 rows. In the case of Sevier 60 the range was from 4.50 to 15.41 per cent and the mean was 7.40 per cent.

For the homozygous dense group the range in the calculated coefficients of variability was from 7.46 to 13.14 per cent, with a mean of 10.34 per cent. In the heterozygous group the range in the calculated coefficients of variability was from 24.82 to 52.30 percent, with a mean of 33.27 per cent. The mean for the homozygous lax group was 7.45 per cent, with a range from 4.55 to 10.71 per cent. In family 5 the ranges and the means of the coefficients of variability were essentially the same, the means being 6.87 per cent for Federation, 7.16 per cent for Sevier 60, 9.90 per cent for the homozygous dense segregates, 33.70 per cent for the heterozygous, and 7.47 per cent for the homozygous lax segregates.

TABLE 13.—*Spike density classes of the means of Sevier and Federation parental rows and of the F_3 families and coefficients of variability classes (C. V.) of the individual rows of the Sevier and Federation parents and of three groups of hybrids—(1) those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 4; grown in 1925 at Logan, Utah]

Strain	Number of plants in spike-density classes											Total	C. V. classes
	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75		
Federation							12	3				15	6
							4	1				5	9
Total							16	4				Mean.	6.57
Sevier 60					13	1						14	6
					4							4	9
					1							1	12
					1							1	15
Total					19	1						Mean.	7.40
Homozygous dense		2										2	6
	3	4	1									8	9
	2	5	2									9	12
Total	5	11	3									Mean.	10.34
Heterozygous						1						1	24
					4	1						5	27
				1	3	3						7	30
				1	1	1						3	33
			1	1	1	1						4	36
					3	1						4	39
				1								1	42
												0	45
												0	48
					1							1	51
Total			1	4	13	8						Mean.	33.27
Homozygous lax							1	2	8	5	1	17	6
								3	6	1	1	11	9
										1		1	12
Total							1	5	14	7	2	Mean.	7.45

TABLE 14.—*Spike density classes of the means of Federation and Sevier parental rows and of the F_3 families and coefficients of variability classes (C. V.) of the individual rows of the Sevier and the Federation parents and of three groups of F_3 hybrid families—(1) those homozygous for dense spikes, (2) those heterozygous for spike density, and (3) those homozygous for lax spikes*

[Family 5; grown in 1925 at Logan, Utah]

Strain	Number of means in spike-density classes											Total	C. V. classes
	1.75	2.25	2.75	3.25	3.75	4.25	4.75	5.25	5.75	6.25	6.75		
Federation						1	14	1				16	6
						1	4	3				8	9
Total						2	18	4				Mean.	6.87
Sevier 60					1							1	3
				2	14	1						17	6
					4							4	9
				1								1	12
					1							1	15
Total				3	20	1						Mean.	7.16
Homozygous dense		2	3									5	6
	1	27	8									36	9
	1	11	3									15	12
	1		1									2	15
												0	18
		1										1	21
Total	3	41	15									Mean.	9.90
Heterozygous							1					1	21
				1	1		1					3	24
				3	6	4						13	27
			1	8	16	6						31	30
				6	12	6						24	33
			1	2	11	7						21	36
			2	5	8	1						16	39
				6	3							9	42
			1	3								4	45
			1									1	48
Total			6	34	57	24	2					Mean.	33.70
Homozygous lax									1			1	3
						1	1	9	12	5	4	32	6
						3	4	8	9	6	1	31	9
												0	12
								1				1	15
Total						4	5	18	22	11	5	Mean.	7.47

The calculated coefficient of variability for each of the F_3 families shows which are breeding relatively true and which are segregating for spike density. The difference between mean coefficients of variability of 10 per cent for those breeding true and 33 per cent for those segregating is so great as to make certain which rows are homozygous or nearly so.

In family 5 grain of each of the F_2 plants was sown. Of the 247 F_3 families, 59 bred true for dense, 64 for lax, and 123 segregated for density (see fig. 4). Table 15 gives the calculation for closeness of fit on a one-factor difference for spike density.

TABLE 15.—*Closeness of fit of three groups of F_3 families compared with the 1:2:1 segregation*

[Family 5; grown in 1925 at Logan, Utah]

Group	C	O	C–O	$(C-O)^2$	$\frac{(C-O)^2}{C}$
Homozygous dense	61.75	59	2.75	7.5625	0.1224
Heterozygous	123.50	123	.50	.2500	.0020
Homozygous lax	61.75	64	2.25	5.0625	.0820
				$X^2=0.2064$	

Since X^2 is less than 1, the probability is very great that the proposed hypothesis is essentially correct. Family 5, in which 247 F_3 families were measured, shows a very close approximation to a

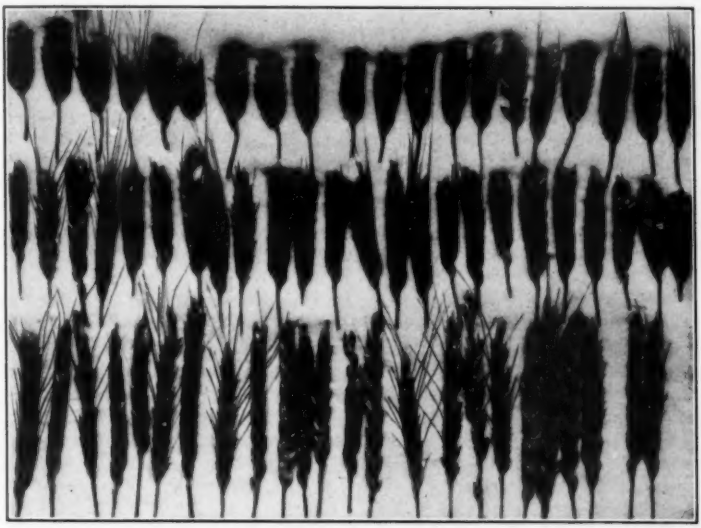

FIG. 4.—Single spikes of each F_2 plants of family 4 in the cross between pure lines of Federation and Sevier. When tested in F_3 all the forms in the top row proved homozygous for dense spikes; those in the bottom row homozygous for lax spikes, and those in the middle row heterozygous for density. There was a very close approximation to a ratio of 1 dense : 2 heterozygous : 1 lax, indicating one major factor difference for spike density. There are in each major group smaller inherited differences in spike density, which indicate that in addition to the one major factor there is a series of minor factors involved in the inheritance of spike density

1:2:1 ratio. This suggests that one main factor is involved in the inheritance of spike density.

That spike density in the Sevier × Federation cross has involved, in addition to one main factor, several minor factors is evident from the fact that one parent was not recovered at all. By referring to Figure 3 (bottom), it can be seen, however, that the least dense family of the homozygous dense segregates is so near to the most dense row of Sevier that if the 10 to 15 per cent variation due to soil heterogeneity be allowed for, it might be considered that there was one F_3 family in which the density of Sevier was recovered. Since there were 247 F_3 families in family 5 and 74 in family 4, the number of segregates breeding true to the spike density of the Sevier parent is so small as to make clear that several minor factors are involved.

This is also borne out by the range of mean densities of the homozygous dense and homozygous lax families. A summary of the range of densities in the parents and in the F_3 families is given in Table 16.

TABLE 16.—*The range of mean spike densities and the mean of mean spike densities of Federation and Sevier rows and of three groups of F_3 families and coefficients of variability (C. V.) of the mean spike densities of Federation and Sevier rows and of F_3 families*

Parent or segregates	Range of spike densities of Federation and Sevier parents and of F_3 families		Mean spike density of parents and F_3 families		C. V. of mean spike densities of parents and F_3 families	
	Family 4	Family 5	Family 4	Family 5	Family 4	Family 5
Federation	4. 567–5. 251	4. 055–5. 237	4. 880	4. 752	3. 28	5. 47
Sevier	35. 92–40. 12	3. 135–4. 022	37. 58	37. 21	2. 45	5. 47
Homozygous dense	1. 945–2. 782	1. 952–2. 825	2. 265	2. 325	10. 50	16. 67
Heterozygous	2. 925–4. 495	2. 437–4. 502	3. 781	3. 688	10. 63	10. 40
Homozygous lax	4. 732–6. 911	4. 110–6. 794	5. 869	5. 600	7. 10	10. 63

The coefficients of variability of the mean densities of the 20 parent rows sown in the family 4 plot were only 3.28 per cent for Federation and 2.45 per cent for Sevier 60 as compared with 10.50 per cent for the homozygous F_3 families and with 7.10 per cent for the homozygous lax families. The same figures for family 5 are all noticeably larger, probably because of greater soil heterogeneity resulting from the fact that the larger number of rows covered about three times as large an area of land. The figures for family 5 are 5.47, 5.47, 16.67, and 10.63 per cent, respectively.

On an average, the variability of the means of the homozygous F_3 families is about 2 to 3 times as great as that of the 20 to 24 parent rows planted at frequent intervals. This, added to the fact that there is a series of small steps throughout the range of variation in mean spike densities, points rather strongly to a series of minor factors which modify the expression of the one major factor for spike density.

As yet there has been found no genetic hypothesis that will explain the fact of wide transgressive segregation of spike density in both directions and still account for the very infrequent recovery of the spike density of one of the parents.

SQUAREHEADEDNESS

The squareheadedness of the 74 F_3 families of family 4 which were grown was obtained by calculating the ratio of the number of rachis internodes in the middle third of the rachis to the number in the upper third. This calculation was made for 20 rows of about 40 plants each of the two parents as well as for the F_3 families. The frequency with which rows of the Federation and the Sevier parents and of the F_3 families occurred when put in classes according to mean squareheadedness and in relation to their calculated coefficients of variability is given in Table 17. The means of the squareheadedness of Federation ranged from 0.99 to 1.17, with a range in the coefficients of variability of from 9.59 to 19.43 per cent, the mean being 14.84 per cent. The range in the mean squareheadedness of the rows of Sevier 60 was from 0.77 to 1.08. The range in the coefficients of variability was from 10.85 to 21.88 per cent, with a mean of 15.30 per cent. The mean squareheadedness of the F_3 families ranged from 0.90 to 1.30. Their smallest coefficient of variability was 10.13 per cent and the largest 29.24 per cent. The mean was 15.81 per cent.

TABLE 17.—*Frequency distribution of rows of the Federation and the Sevier parents and of 74 F_3 families arranged in classes according to the mean squareheadedness and according to the size of the coefficients of variability*

[Family 4; grown in 1925 at Logan, Utah]

Strain	Distribution of rows according to classes of squareheadedness																			Total	C. V. classes
	.78	.81	.84	.87	.90	.93	.96	.99	1.02	1.05	1.08	1.11	1.14	1.17	1.20	1.23	1.26	1.29			
Federation														1						1	9
										1		2	2	1						6	12
									1	1	2		1	2						7	15
								1		1	2		1	1						6	18
Total								1	1	3	4	2	4	5						Mean.	14.84
Sevier 60						3	1	1												5	12
				2		2				1	1									7	15
				1	2	1														4	18
	1	1		2																4	21
Total	1	1		5	2	1	6	1	1	1	1									Mean.	15.40
F_3 families						1			1											1	9
									2	1	4	5				1				14	12
				3		2	3		6	5	5	3	3	1	1	1			1	34	15
					1	3	2		2	3	3	1	1	1		1				18	18
									1	1	1				1					4	21
											1									1	24
												1								1	27
											1									1	30
Total					3	2	5	5	12	10	15	10	4	2	2	3		1		Mean.	15.81

The distribution of the rows of each parent indicates a genetic difference in squareheadedness between the Federation and the Sevier parents. The variations in squareheadedness are so great as to make impossible an accurate study of the inheritance of this character. Though the F_3 familes have a somewhat wider absolute range of squareheadedness and of coefficients of variability, the distribution of the F_3 families into each group does not give much greater calculated coefficients of variability than those for the parents. No correlation for squareheadedness was found between F_2 plants and F_3 families.

TALLEST CULM

The plants in the F_3 families of family 4, together with the plants from the respective rows of each parent, were measured in order to ascertain the length of the tallest culm. The average height of the shortest row of Federation was 92.2 cm. and that of the tallest 106.3 cm., the mean height being 99.8 cm. For Sevier 60 the average height of the shortest row was 112.8 cm., and that of the tallest 126.3 cm., with a mean height of 119.1 cm. For the 74 F_3 families the mean height of plants in the shortest family was 99.0 cm. and that of the tallest family 135.3 cm., with a mean height of 111.4 cm.

The frequency distribution of the means of both parents and of the means of the 74 F_3 families when grouped in classes according to the heights of plants and the coefficients of variability is shown in Table 18. From the table it is apparent that there was a distinct difference in the length of the tallest culms of the two parents. The means of culm lengths in F_3 families were more variable than those of either parent. This is shown by the fact that the coefficient of variability of the means of the F_3 lines is 5.29 per cent, whereas the mean coefficient of variability of Federation is 4.54 per cent, and that of Sevier 3.63 per cent.

TABLE 18.—*Frequency distribution of rows of the Federation and the Sevier parents and of 74 F_3 families arranged into classes according to height of the tallest culm in centimeters and according to the size of the coefficients of variability*

[Family 4; grown in 1925 in Logan, Utah]

Strain	Rows in classes based on height of tallest culm															Total	C. V. classes
	93	96	99	102	105	108	111	114	117	120	123	126	129	132	135		
Federation			1		2											3	3
		2	3	3	1											9	4
		2	1	1												4	5
		1			1											2	6
	1		1													2	7
Total	1	5	6	4	4											Mean.	4.54
Sevier 60										1						1	2
								1		1	3	1				6	3
								5	2	1	2	2				12	4
								1								1	5
Total								7	2	3	5	3				Mean.	3.63
F_3 families					2	2	2	3	2	2	3				1	17	4
			1		5	6	7	3	3	1	1					27	5
			1	1	4	7	2	5	2		1					23	6
					1		2	1			1					5	7
					1											1	8
																0	9
						1										1	10
Total			2	1	13	16	13	12	7	3	6				1	Mean.	5.29

The distribution of the means of the parental rows in classes according to the average height of the plants in relation to coefficient of variability classes indicates a genetic difference between the Federation and the Sevier parents. The mean heights of the F_3 families range from a little more than the average height of the shortest row of Federation to somewhat more than the average height of the tallest row of Sevier.

These results indicate that there was segregation for this character, but that its nature can not be accurately determined from the data available.

CORRELATION STUDIES

A study was made of F_2 plants to find out whether certain plant characters were correlated. The observations on each plant were taken separately, and later the data relating to each plant were copied on separate cards. This permitted sorting the cards into groups according to the characters being correlated. Forty-nine tables were made in connection with the 8 families, for each of which the coefficient of correlation (r), the correlation ratio (η), and Blakeman's test for linearity were calculated. These constants are given in Table 19.

Spike density has been shown to be a definitely inherited character dependent upon one major factor. The first part of Table 19 gives the correlations of spike density with other F_2 plant characters.

In 4 families the correlation between spike density and the length of the longest culm was studied. The r's are all small, partly − and partly +, and indicate an absence of correlation such as r will measure. On the other hand, the η's all approach 0.2 to 0.3, which

figures are from 4.7 to 9 times the probable errors, and suggest that some sort of correlation exists. Since the values of three of the four Blakeman's tests for linearity are greater than 3 and the other one is greater than 2, the chances seem to be that there is a small correlation, with about 1 chance in 20 of its being linear.

TABLE 19.—*Correlation coefficients* (r), *correlation ratios* (η), *their respective probable errors* (*P. E.*), *and Blakeman's test of linearity for various pairs of plant characters*

[F_2 individual plants, grown in 1924 at Logan, Utah]

Characters related, and family	Number of plants	$r \pm P.E.$	$\frac{r}{P.E.}$	$\eta \pm P.E.$	$\frac{\eta}{P.E.}$	Blakeman's test
Spike density×longest culm:						
Family 4	393	−.059±0.34	1.7	0.241±.032	7.6	3.4325
Family 6	246	−.027±.043	.6	.193±.041	4.7	2.2285
Family 7	301	+.079±.039	2.0	.315±.035	9.0	3.9177
Family 8	239	−.128±.043	3.0	.295±.040	7.4	3.0278
Spike density×number of culms:						
Family 4	393	−.108±.034	3.2	.196±.033	5.9	2.4033
Family 5	248	+.035±.043	.8	.223±.041	5.4	2.5673
Spike density×total length of culms:						
Family 4	393	−.043±.034	1.3	.205±.033	6.2	2.9365
Spike density×awn classes:						
Family 4	393	−.020±.034	.6	.118±.034	3.5	1.7253
Family 5	248	−.079±.043	1.8	.228±.041	5.6	2.4996
Family 6	247	−.035±.043	.8	.223±.041	5.7	2.6798
Family 7	301	+.131±.038	3.4	.150±.038	3.9	.9556
Family 8	239	−.014±.044	.3	.211±.042	5.0	2.3518
Spike density×thickness of neck:						
Family 5	248	−.046±.043	1.1	.355±.037	9.6	4.1151
Spike density×square-headedness:						
Family 1	349	−.545±.025	21.8	.573±.024	23.9	.2527
Family 2	173	−.502±.038	13.2	.602±.033	18.2	3.2362
Family 3	156	−.591±.035	16.9	.628±.033	19.0	1.9611
Family 4	393	−.542±.024	22.6	.615±.021	29.3	4.2739
Family 5	248	−.542±.030	18.1	.570±.028	20.4	2.0510
Family 6	247	−.515±.032	16.1	.608±.028	21.7	3.7538
Family 7	301	−.463±.031	14.9	.578±.026	22.2	1.8081
Family 8	239	−.503±.033	15.2	.544±.031	17.5	2.3824
Awn classes×spike shape (eye classification):						
Family 4	393	+.042±.034	1.2	.070±.034	2.1	.8141
Awn classes×longest culm:						
Family 4	393	−.038±.034	1.1	.115±.033	3.5	2.1619
Family 6	247	+.019±.043	.4	.205±.041	5.0	2.3815
Awn classes×number of culms:						
Family 4	393	+.112±.034	3.3	.160±.033	4.8	1.6822
Family 6	247	−.037±.043	.9	.217±.041	5.2	2.4865
Longest culm×total length of culms:						
Family 4	393	+.530±.024	22.1	.564±.023	24.5	2.8364
Longest culm×number of culms:						
Family 4	393	+.340±.030	11.3	.392±.029	13.5	2.8673
Square-headedness×longest culm:						
Family 1	349	−.054±.036	1.5	.330±.032	10.3	4.5138
Family 2	173	+.137±.050	2.7	.306±.046	6.7	2.6746
Family 3	156	+.240±.051	4.7	.649±.031	20.9	5.6084
Family 4	393	−.029±.034	.8	.208±.033	6.3	3.0247
Family 7	301	−.035±.039	.9	.265±.036	7.4	3.3725
Family 8	239	+.020±.044	.5	.331±.039	8.5	1.1775
Square-headedness×thickness of neck:						
Family 1	349	+.153±.035	4.4	.339±.032	10.6	4.1853
Family 2	173	+.116±.051	2.3	.318±.046	7.0	2.8885
Family 3	156	+.248±.051	4.9	.301±.050	6.0	1.5564
Family 5	248	+.212±.041	5.2	.315±.039	8.1	2.7075
Family 6	247	+.147±.042	3.5	.330±.038	8.7	3.4382
Family 7	301	+.146±.038	3.8	.196±.037	5.3	1.6964
Family 8	239	+.077±.043	1.8	.199±.042	4.7	2.0849
Square-headedness×awn classes:						
Family 1	349	−.028±.036	.8	.133±.036	3.7	1.7943
Family 2	173	−.063±.051	1.2	.221±.049	4.5	2.0591
Family 3	156	−.084±.053	1.6	.269±.050	5.4	2.3736
Family 4	393	−.110±.034	3.2	.155±.033	4.7	1.5948
Family 5	248	−.024±.043	.6	.166±.042	3.9	1.9235
Family 6	247	−.020±.043	.5	.255±.040	6.3	2.9671
Family 7	301	−.139±.038	3.7	.414±.032	12.9	5.0088
Family 8	239	−.011±.044	.3	.141±.043	3.3	1.5646

Spike density and number of culms seem to show a slight correlation as measured by the η's, which are 0.196 and 0.223, values which are more than five times their probable errors. The two r's are small and contradictory, and the two Blakeman's tests indicate that the chances are 1 in 4 that the correlation is linear.

The r for spike density correlated with total length of culm was -0.043, scarcely larger than its probable error. The η is 0.205, which is 6 times its probable error. This apparently indicates a small correlation. Since the Blakeman's test is essentially 3, the chances are about 1 in 20 of its being linear.

The r's for spike density and awn classes are small, some plus and some minus. The η's vary from 0.1 to more than 0.2 and from three and five-tenths to more than five times their probable errors. The values of η are small, but seem to suggest the likelihood of a small correlation. The five Blakeman's tests vary from 0.9 to 2.6, values which suggest linearity. Such will be the case, however, when the η is very small even if r is essentially 0.

In the one correlation study of spike density and thickness of neck, r is too small to be significant, but η is 0.355, which is nine times its probable error. This is a fairly strong indication of a correlation. The fact that the value of Blakeman's test is more than 4 indicates that the chance of linearity is 1 in 142 trials. This suggests nonlinearity.

The coefficients of correlation of spike density $\times$squareheadedness are all above 0.5 and are from about 13 to more than 20 times the probable errors. The η's are all 0.6 or nearly so and from 17 to 23 times the probable errors. Five of the eight Blakeman's tests are less than 3, and three are between 0.2 and 2, indicating linearity. All these figures seem to indicate a strong tendency of the more dense spikes to be more squareheaded.

Since, however, squareheadedness was so very greatly influenced by environment that the nature of its inheritance was not determined, it can not be safely said that even these large figures indicate linkage.

When awn classes and spike shape as determined by eye classification were correlated, r and η were both essentially 0, and neither was significant in the light of its probable error, indicating absence of correlation between these two characters.

In the correlation between awn classes and the number of culms, the η's are about five times their probable errors and suggest some correlation, though one r is plus and the other minus. The values of the two Blakeman's tests for linearity are one less than 2 and the other more than 2. The chances are about 1 in 4 that there is linearity in the correlation.

In the correlation between awn classes and length of the longest culm in the two families represented, the r's are very small and contradictory. One η is five times its probable error and the other one three and five-tenths times. These may indicate some correlation. The values of the two Blakeman's tests for linearity indicate that there is 1 chance in 5 or 6 of the correlation's being linear.

One would expect to find a high correlation between the length of the longest culm and the total culm length. Both r and η are greater than 0.5 and more than 20 times the probable error. It may be that

this figure is an additive result of plant vigor and of correlating two things one of which is part of the other.

In the one family studied, the length of the longest culm and the number of culms seem to be positively related since r is $+0.340$ and is 11 times its probable error; η is 0.392 and 13 times the probable error. Blakeman's test for linearity is less than 3 and also suggests linearity. It may be, however, that vigorous plants would tend to produce both more culms and longer ones. In so far as vigor and length of culm are due to the same genetic factor, even with the fair-sized constants, the correlation would be physiologic rather than genetic.

When squareheadedness is correlated with the length of the longest culm, with the thickness of the neck, and with awn classes, most of the r's are small and the η's fairly good sized and 3 to 20 times their probable errors. If squareheadedness were heritably a stable character, it is likely that constants of the magnitude obtained would indicate correlations. In view of the great influence of environment on the variability of squareheadedness in the material here used, it does not seem wise to ascribe much genetic importance to these figures.

SUMMARY

In a variety cross of Dicklow×Sevier wheats, some F_3 and F_4 families considerably more compact of spike than either parent and some considerably more lax than either parent were found to breed true. In the nursery test some of the homozygous lines gave yields sufficiently higher than that of the high-yielding parent to appear promising from the standpoint of wheat improvement.

About 50 of the homozygous lines from the Dicklow×Sevier cross were tested at University Farm, St. Paul, Minn., in 1924 for resistance to *Puccinia graminis tritici*. Two of the lines (G 40 and G 149) were found to be very resistant in the field. Seedling plants of these two lines and of both the parents were inoculated in the greenhouse with all the physiologic forms of black stem rust then available. The Dicklow parent was completely susceptible to every form tested. The Sevier parent was somewhat resistant. G 40 and G 149 proved resistant, G 149 was highly resistant to many forms and at least moderately resistant to all of the 19 forms available for testing.

In 1925, G 40 and G 149 were grown in the uniform rust nurseries in various parts of the United States and Canada and proved to be as resistant as the most highly resistant varieties of vulgare wheat known.

A detailed genetic study was made of a cross between pure lines of Federation and Sevier wheats, which were contrasted as follows:

Federation	*Sevier*
Awnless.	Awned.
Spike density intermediate.	Spike density intermediate, but a little more dense than that of Federation.
Slightly squareheaded.	Not squareheaded.
Short culms.	Long culms.

In F_2 a study was made of individual plants. Four classes of awns were defined, and each plant was classified by comparison with mounted specimens showing the range of variability for each class.

Measurements were taken of the length of the rachis, the length of 10 rachis internodes, the thickness of the neck just below the spike, and the length of the culms; and counts were made of the number of spikelet internodes in the middle third of the rachis and in the upper third (as a basis for calculating the ratio of squareheadedness) and of the number of culms.

In F_3 the F_2 genotypes were tested by the breeding behavior of F_3 families, each from a single F_2 plant. One row each of the Federation and the Sevier parents was sown among the F_3 families after every 4 hybrid rows in family 4 and after every 10 hybrid rows in families 5 and 6. Mother plants for the 74 F_3 progenies of family 4 were chosen at random from plants represented on each of the 12 sections of the spike-density curve of family 4. Grain of all the F_2 plants of families 5 and 6 was sown.

At harvest the F_3 progenies of family 4 were studied to determine awn classes spike density, squareheadedness, and length of culm. The 247 F_3 progenies of family 5 were studied to determine spike density and awn classes, and the awn classes of the 245 F_3 progenies of family 6 were determined.

Four homozygous classes of awns were obtained, one of each parental type and two intermediate types. In all three families the two parental classes were considerably more numerous. This fact suggested linkage, and when the observed proportions of homozygous and segregating progenies were studied by the closeness-of-fit method it was found that the hypothesis that there are two factors for awns located in the same chromosome and that there is 35 per cent crossing over explains the results.

The F_2 plants, as tested by the F_3 breeding behavior, gave a close approximation to a 1:2:1 ratio of dense, intermediate, and lax spikes. Approximately one-fourth of the total families had dense spikes and one-fourth had lax spikes. Both of these groups of families proved homozygous, and the intermediates all segregated.

In each of the three major groups there was a series of smaller differences in spike density, as is shown by the fact that the coefficients of variability of the means of the spike densities in the three major groups of F_3 families were considerably greater than were the coefficients of variability of the rows of either parent. The spike density of the Sevier parent was obtained again in not more than one of the 321 families tested and perhaps not all. Therefore, one major factor and a series of minor factors are involved in the inheritance of spike density in the Federation x Sevier cross.

The ratio of squareheadedness was found to be distinctly different in the Federation and in the Sevier parents. The ranges in the individual rows of the parents and in the F_3 progenies showed clearly an enormous variation in this character, due to environment. The coefficients of variability were approximately 15 per cent.

In order to find out the nature of inheritance of squareheadedness it would therefore be necessary to reduce variability due to environment by growing F_3 and F_4 families in replicate rows in various parts of the field adjacent to the parents.

The average height of Federation was distinctly less than the average height of Sevier. The F_3 families gave a range of mean heights about equal to the ranges of both parents combined. There

was definite evidence that segregation had taken place as regards height of plant, but the nature of the segregation could not be determined accurately. This could be studied by growing replicate rows of hybrids and parents in various parts of the field.

In correlation studies of F_2 plant characters, r, η, and Blakeman's test of linearity were calculated. Small but apparently significant correlations as judged by their probable errors were found between spike density and the length of the longest culm, between spike density and the number of culms, between spike density and the total culm length, between spike density and awn classes, between spike density and thickness of neck, between awn classes and the length of the longest culm, and between awn classes and the number of culms.

The odds were about 142 to 1 that the correlation between spike density and thickness of neck was nonlinear. In all the other cases the odds were rather small and suggest that the other correlations are a reasonable approach to linearity, as judged by the number of series calculated.

Squareheadedness correlated with spike density gave rather large r's and η's. When squareheadedness was correlated with awn classes the η's were 3 to 12 times their probable errors. However, in view of the great variability of the squareheadedness character due to environment, no great genetic importance was attached to these correlations.

Rather large r's and η's were found in the correlation study between the length of the longest culm and total culm length and between length of the longest culm and the number of culms. These correlations were thought to be physiologic rather than genetic in their nature.

In view of the consistently significant correlations, as judged by their probable errors, between the two stable and definitely inherited characters of spike density and awn classes, it seems reasonable to conclude that there is a strong suggestion of linkage between the factors for these two characters.

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